
Sequencing the OAS 1 gene to map variation

NAME: JANARTH NALVELNATHAN

Degree: Mres Neuroscience

Supervisor: Dr Kevin Morgan, Mr Joern Steinert

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Abstract

Oligoadenylate synthase 1 (OAS1) has been reported to be a key player in the chance of contracting COVID-19 and in the risk of Alzheimer's Disease. The presence of the risk Single Nucleotide Polymorphisms (SNPs) rs1131454 in the 3rd exon, resulting in a substitution of the (GG) ancestral genotype to an (AA) genotype, has been shown to have an association with both AD and COVID-19. Exonic information has been sequenced and well documented, however intronic data has not. This study aims to sequence the OAS1 introns, exons and record potential novel rare SNPs present within as well as confirmed SNPs.

Using 12 individual DNA samples, 4 DNA samples with AD that were homozygous with the risk allele, 4 control samples that were homozygous with the non-risk allele, and 4 control samples that were homozygous with the risk allele, we use PCR techniques to amplify the desired OAS1 locus and sequence the amplicons using next-generation sequencing techniques (Deepseq). We identify a possible novel and known SNPs present on both exonic and intronic data of the OAS1 gene. This potentially novel SNP is located on chr12:112905116 and substitutes the T ancestral base with the C base. It was sequenced in a homozygote control sample with the non-risk allele with a Qscore of 16.64%. Rs7135577, Rs1051042 and Rs10774671, SNPs that are documented to be linked to West Nile Virus (WNV) and Tuberculosis (TB), were recorded in all risk allele samples with Qscores of over 30%, and most likely to be positive SNPs.

Conclusively, our data shows a potential novel SNP on chr12:112905116 with future implications to find a potential link between it and AD, as well as multiple known SNPs that are linked to TB and WNV. Future studies can explore the risk factor and susceptibility to AD with the aforementioned SNPs.

1. Introduction

1.1 Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of dementia. Accounting for 60 to 80 % of dementia cases, and commonly resulting in cognitive ability impairment and memory loss (statistics found from the Alzheimer's Association website: <https://www.alz.org/alzheimers-dementia/what-is-alzheimers>, accessed in Oct 2021). Predominantly seen in people aged over 65 in the UK, increasing age is considered to be a major risk factor for AD and is not part of normal ageing. More than 520,000 people, and rising, in the UK have been diagnosed with AD (Accessed statistics from the Alzheimer's Society <https://www.alzheimers.org.uk/about-dementia/types-dementia/alzheimers-disease> in Oct 2021).

AD is progressive and worsens over time, symptoms first appearing in the latter part of life, termed Late onset Alzheimer's Disease (LOAD). LOAD may be caused by genetics, but sporadic in nature, with over 50 genes found to be associated (Magusali et al., 2021a) and one gene consistently being found to be involved, Apolipoprotein E (APOE) (Bekris et al., 2010). Having the risk allele APOE ε4 doesn't necessarily mean there is a definite development of LOAD as there are cases of individuals living past 90 carrying this allele (Corrada et al., 2013). This suggests environmental and genetic factors coincide with one another in the development of LOAD. Early onset Alzheimer's disease (EOAD) is also existent, but not as prevalent as the former, representing less than 10% of all cases under 65 years are EOAD (Mendez, 2017). EOAD has a higher heritability in comparison to LOAD estimated to be above 90% (Wingo et al., 2012), caused by autosomal dominant mutations in the following three genes: Amyloid Precursor Protein (APP), Presenillin1 (PSEN1) and Presenillin2 (PSEN2). However epidemiologic evidence suggests that only a select few of EOAD cases result from dominant alleles, whilst the majority are unexplained (Campion et al., 1999). This

puts forward the idea that maybe there is another causal factor, that is yet to be explored. Recessive allele, as opposed to dominant allele, was a factor that was considered in a recent study (Wingo et al., 2012). With a bigger proband size than other studies that explored EOAD (Rao et al., 1994; Farrer et al., 1990), this study suggests that recessive alleles can also be a factor. The main difference being the sample demographic used are earlier onset cases, as a key study design change, compared to other studies. A concordance of about 50% was expected between parent and offspring if EOAD was mostly autosomal dominant. The results showed there was an overall concordance of <10%, rejecting the idea that EOAD is entirely autosomal dominant based.

Numerous hypotheses have been suggested for the pathology of AD, ranging from the explanation of the tau and amyloid hypothesis, cholinergic neuron damage hypothesis and Oxidative stress. The extracellular build-up of amyloid plaques is mainly composed of amyloid beta protein, which is excised and released into the membrane from Amyloid precursor protein by beta and gamma secretases. It divides into a short and long amino acid chain (Kametani & Hasegawa, 2018). In control subjects, these chains would degrade or be removed. Under pathological conditions, in aged subjects the metabolic degradation of these chains is decreased, hence the accumulation of Amyloid plaques. These soluble beta-amyloid protein monomers form a dense insoluble and neurotoxic plaque which in turn leads to the causation of Alzheimer's disease through neuronal dysfunction and neuronal death (Tiwari et al., 2019).

Several studies have been conducted using genetically modified mouse models, in which Amyloid beta is deposited in the brain. Plaque formation was seen, however this did not lead to nerve cell death (Bryan et al., 2011). This suggested that the accumulation of Amyloid beta is not cytotoxic. Whilst the majority of AD research uses murine models showing increased Amyloid beta levels, there are numerous other factors associated with AD pathology that are not represented by these models.

The detection of accumulated Amyloid beta in patients' brains has been made easier with recent advancements in amyloid imaging. It is possible to observe control individuals who have amyloid plaques and also AD individuals with lower than expected amyloid accumulation (Edison et al., 2007; Li et al., 2008). Amyloid beta deposition could be a result of ageing and may not be of direct influence on the pathology of AD.

In conjunction to amyloid beta and tau neurofibrillary tangles (NFTs) work together, independently of their accumulation into plaques and tangles, to turn healthy neurons into a corrupted state within the brain. NFTs are made up of unique and twisted fibrils with paired helical filaments of hyperphosphorylated tau protein. Tau protein regulates the stability of tubulin, helping with the stabilizing of the cytoskeleton of the neurons in the brain. The human tau gene locus is situated on chromosome 17 (Spillantini et al., 2000). Exon 10 of this gene encodes for the microtubule binding region. Tau isoform 4repeat (4R) is expressed with the insertion of exon 10, and 3 repeats (3R) tau isoform is produced without exon 10. Both 3R and 4R are expressed in adult human brains, under normal physiological conditions. In AD individuals, 3R and 4R are accumulated in a hyperphosphorylated state. The disarray in the state of microtubules, with the accumulation of aggregated NFTs (Kolarova et al., 2012) along the axon of neurons causes synaptic loss and a potential correlation to AD (Gómez-Isla et al., 1997).

Cognitive Impairment in AD has been considered to be a by-product of pathological changes occurring from damaged cholinergic neurons in the cerebral cortex of individuals with AD (Whitehouse et al., 1982). Cholinergic neurons use Acetylcholine (ACh), which is involved in important physiological systems such as memory, learning, attention, sensory information and stress response (Dunnett et al., 1991; Sarter & Bruno, 1997). The nucleus basalis of Meynert (NbM) was identified as the primary source of cortical cholinergic stimulation for memory in the primate brain (M. M. Mesulam & Van Hoesen, 1976). The cholinergic hypothesis was a consideration, this involves the testing of cholinesterase inhibitors on AD individuals, which results in symptomatic improvements in individuals

(Summers et al., 1986). This was done through a three-phased trial on moderate to severe AD patients with oral tetrahydroaminoacridine (THA). THA treatment produced better results than its placebo counterpart, putting forward the idea that cholinesterase inhibitors can be temporarily useful in treating AD. Neurofibrillary degeneration of Cholinergic NbM neurons was consequently found to be a diagnosis in the early stages of AD and was correlated with the deficits of cognition (M. Mesulam et al., 2004; Mufson et al., 2008). Tacrine was the first anti-AD cholinesterase inhibitor drug used in clinics. It showed severe side effects and produced no clear results between Tacrine and the placebo group. It did not show any significant benefit for AD participants and therefore it was no longer available on the market (Qizilbash et al., 2000). Other cortical neurotransmitters such as dopamine, norepinephrine and histamine were documented in AD, however it was concluded that cholinergic lesions were more consistent in the alterations of AD compared to other neurotransmitters (Bartus et al., 1982; Drachman & Leavitt, 1974).

Another important pathological aspect of AD is Oxidative stress. Oxidative stress is the by-product of a contrast between the production and the detoxification of reactive oxygen species (ROS). ROS are crucial in their function in signalling molecules under carefully controlled conditions. However if in excess, they can do significant damage to nucleic acid, proteins, and lipids, as they are capable of oxidizing these major biomolecules (Ferrer et al., 2010). Due to the brain's high energy consumption and oxygen consumption, it is prone to oxidative imbalance. Studies have reported extensive damage to biomolecules and evidence to suggest that oxidative stress does indeed play a role in AD (Nunomura et al., 2001). The levels of 8-hydroxyguanosine, a biomarker for detecting oxidative stress, was higher in early events in AD and decreased over time with disease progression. Increase in Amyloid beta deposition was seen to be correlated with the decrease in damage derived from oxidative stress. Anti-oxidant compound treatments would in theory provide sufficient protection and delay the onset whilst also reducing the progression of AD (Velagapudi et al., 2018). However, this approach has not been thoroughly studied yet.

These hypotheses are very well accepted and are used as a basis for drug research development. Beta-Amyloid Immunisation has been explored as a stratagem against AD. Vaccination is considered to be extremely effective against infectious diseases (Rodrigues & Plotkin, 2020). It is also feasible to consider vaccinations to work against neurodegenerative diseases. Anti-beta-amyloid Antibodies, a protein designed to counteract beta-amyloid dependent neurodegeneration, have been shown to interfere with beta-amyloid aggregation blocking beta-amyloid toxicity and decreasing beta-amyloid plaque levels in the brain. Documentation of peripherally induced antibodies in mouse models of Alzheimer's Disease has shown a reduction in amyloid levels (Bard et al., 2000). 8-10 months old Heterozygous platelet-derived APP mice were induced with polyclonal immunoglobulin obtained from mice that were immunized with 42 amino acid form of Amyloid beta (pabA β ₄₂). After weekly injections, the plaque deposition was reduced by 81% relative to control mice. However these were only documented using murine model analysis, and therefore the results may be different with a human model.

In a more recent study, this was observed with the human monoclonal antibody, Aducanumab, which targets beta Amyloid aggregate exclusively by removing it from the brain (Sevigny et al., 2016). However, it was unclear whether the amyloid clearance protects individuals from cognitive decline (Walsh et al., 2021). Biogen, a biotechnology company, introduced the Engage and Emerge clinical study trial, this 18-month study aimed to compare the effects of 3 doses of aducanumab against a placebo. Phase III trial for this antibody was discontinued due to having failed to show strong evidence compared to the placebo group (Mukhopadhyay et al., 2021). The reason behind this conclusion was primarily due to the Engage study having failed, whilst the Emerge study concluded promisingly. Biogen concluded that the trials were not futile, and resulted in further findings with the conclusion that this antibody decreases the amyloid levels in the brain and phosphorylated Tau levels in the cerebrospinal fluid (Knopman et al., 2021).

Similar research on the medication Lecanemab, another monoclonal antibody that acts by blocking Amyloid aggregate formation, has been approved by the FDA as a potential treatment for EOAD after released data from Phase 3 clinical trial suggests that there was momentous findings (CH et al., 2023). Lecanemab slowed down the speed at which cognitive skill degraded in people with EOAD, by 27% compared to people who took the “dummy” drug (CH et al., 2023). This medication is an important milestone; however, it only combats EOAD, and is unlikely to affect people with LOAD. The difficulty in diagnosing individuals with EOAD early remains an issue, therefore treating these individuals will still be a challenge for the foreseeable future.

With ever-rising AD cases, its effect on society makes it all the more important to eradicate this neurological condition. Recent studies have highlighted immunology and neuroinflammation, with the involvement of microglial in the brain, the local macrophage, playing a major role in the pathogenesis of AD (Magusali et al., 2021a).

1.2 Macrophage’s role in AD

The central nervous system (CNS) as an immune-privileged organ, hosts numerous populations of myeloid cells and defensive barriers such as the meninges, the perivascular space and the choroid plexus. The microglia, the innate immune cells of the CNS, serve as the phagocytes that play a crucial role in CNS tissue maintenance and response to foreign pathogens and injuries (Colonna & Butovsky, 2017; Nayak et al., 2014). Discoveries of APOE, TREM2, CD33 and other genes all expressed by macrophages, increase the likelihood chance of developing Alzheimer’s disease (Hansen et al., 2018; Verheijen & Sleegers, 2018). These genes primarily act in allowing microglia to respond to amyloid, such cells are termed ARM – Amyloid responsive microglia. It is postulated that there is another form of cell, called the IRM - interferon responsive microglia, and this is where the OAS gene seems to be contributing towards Alzheimer’s disease (Magusali et al., 2021b).

In a few Genome-Wide Association Studies (GWAS) it was shown that several genes IFNAR2, CCHCR1, and OAS were all involved in interferon signalling, which contributed to the genetic risk associated with COVID-19 (Pairo-Castineira et al., 2020; Schmiedel et al., 2020). 11 protein-coding genes were significantly associated with severe COVID-19 risk allele variants, after assessing the effects on 13 different immune cell types. These overlapped with target gene promoters or cis-regulatory areas in cell types where the effects were most prominent. Genotype dependent effects in non-classical monocytes, B cells or T-cells, were shown in the expression of OAS1 and 3 (Schmiedel et al., 2020). This highlights the importance and COVID-19 risk variants, to influence the functions of different immune cell types.

1. 3 Coronavirus

2019 saw the emergence of a novel coronavirus, severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Originating in the Wuhan province of China, it spread rapidly worldwide and resulted in the outbreak of the COVID-19 pandemic. As of March 2021, the estimated death toll was calculated to be around 6 million, with over 400 million confirmed cases worldwide and still rising, causing severe health and economic issues (Statistics taken from the official World Health Organisation website dedicated to coronavirus: <https://covid19.who.int/>).

Intriguingly, SARS-CoV-2 infection does vary in consequences, ranging from asymptomatic to debilitating viral pneumonia and acute respiratory syndromes (Niemi et al., 2021). Host factors such as age and sex do play a role in the severity, however numerous other risk factors may also contribute towards this.

COVID-19 is classified as a respiratory disease; however many patients display a range of symptoms, some of which may be due to the effect of SARS-CoV-2 on the neurological system. The loss of taste and smell, as well as the effect of headaches to anxiety and seizures, can all be a by-product of this system. The disturbance in

the homeostasis of the CNS can ultimately result in neuronal cell death (Mishra & Banerjea, 2020).

The effect of SARS-CoV-2 on the Central nervous system (CNS) could be explained through different mechanisms. SARS-CoV-2's effect on the CNS can firstly be explained by the systemic inflammatory response with the release of large amounts of pro-inflammatory cytokines, also known as a “cytokine storm”. These systemic effects, which are seen in severe cases of COVID-19, also trigger a considerable increase in the circulation levels of chemokines and interleukins exposed at the blood-brain-barrier, thereby entering the brain parenchyma and affecting the immune response through astrocytes and microglia (Tremblay et al., 2020).

Long Covid symptoms could be a potential by-product of the recurrently active microglia after the elimination of the virus (Ohgidani et al., 2017). Chronically active microglia and their persistent active state have been recorded in other neuropsychiatric conditions, like depression (Mondelli V et al., 2017). Long Covid is the term used to describe COVID-19 symptoms in people who have recovered (Yelin et al., 2021) but are still reported to have lasting effects of the infection for far longer than expected (Fotuhi et al., 2020).

Another proposed idea is the entry point of SARS-CoV-2 through Angiotensin Converting Enzyme 2 (ACE2). SARS-CoV-2 enters the host alveoli through the respiratory tract (Zhang et al., 2020). The spike protein present on SARS-CoV-2, binds to ACE2 and damages the vascular endothelium through the downregulation of ACE2 leading to infection in the lungs (Lei et al., 2021).

Recent Genome-Wide Association studies (GWAS) have found that genetic factors may have a role in the susceptibility and severity of COVID-19, providing further insight as to the pathogenesis of this disease, and possibly uncovering analeptic treatments in the future. Despite recent vaccine rollouts, treating this disease is of utmost importance. Numerous loci containing genetic variants that may contribute toward the severity of COVID-19 have been identified using GWAS, one of which is the OAS locus (Severe COVID-19 GWAS Group et al ., 2020).

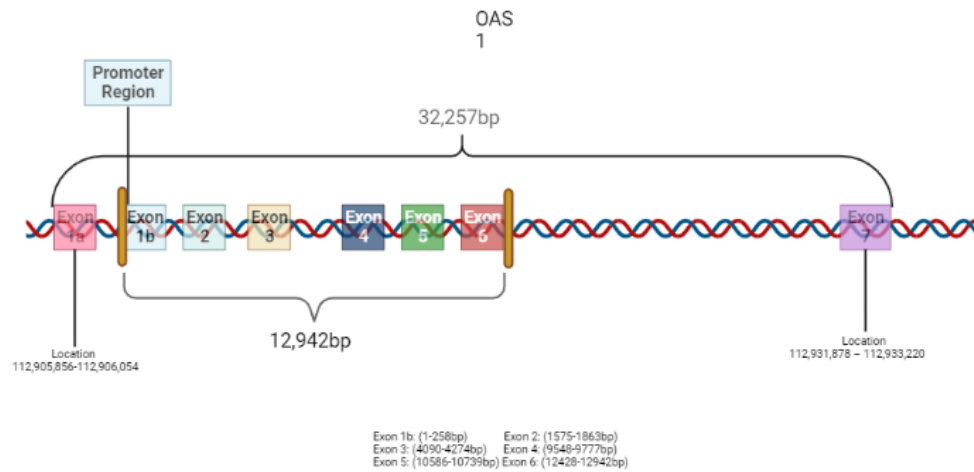
The existence of two variants, SNPs (Single nucleotide polymorphisms) in the OAS locus related to COVID-19, have been confirmed to have Linkage Disequilibrium (LD) adjacent to the SNPs relating to the risk of LOAD (Magusali et al., 2021b). The confirmed SNPs relating to COVID-19 are found on OAS3 (chr12:112942203; rs10735079) and OAS1 (chr12:112925745 ; rs6489867) (Magusali et al., 2021b). These haplotype mutations occur within the OAS locus and therefore suggest that there could be an underlying mechanism which connects both COVID-19 and LOAD. The OAS family of proteins may indeed be a contributing factor in the severity of COVID-19 and the occurrence of Alzheimer's Disease. Confirming these SNPs within the context of this study could validate the LD findings (Magusali et al., 2021b). Recently it has been suggested that the human OAS locus also contains a protective haplotype, passed down from Neanderthals (Zhou et al., 2021), which helps with the negation of contracting COVID-19. Further insinuating the importance of these haplotypes located in the OAS locus and the influence on the risk of people contracting and getting LOAD or COVID-19.

1.4 OAS Family

The 2'-5' *oligoadenylate synthetases* (OAS) consist of a group of interferon-induced enzymes (Magg et al., 2021). They play a vital part in the defence against viral infections (Kristiansen et al., 2011). The activation of these enzymes by cytoplasmic foreign nucleic acids such as double-stranded RNA, causes the oligomerisation of ATP into 2'-5' *oligoadenylate* (2-5A). 2-5A then binds to latent Ribonuclease L (RNase L), causing a decrease in the synthesis of viral protein by cleaving single-stranded RNAs (Ghosh et al., 1991). As a consequence, this triggers the degradation of viral and cellular RNA (Han et al., 2014) (Magg et al., 2021), further inhibiting viral replication.

In *homo sapiens*, the OAS locus is located on the 12th chromosome in the 24.2 position (Rebouillat & Hovanessian, 2004). Spanning 130,000 base pairs in length, it encodes for 4 genes: OAS1, OAS3, OAS2 and OASL appearing in this respective order. They each encompass one, two and three repeat OAS domain units respectively. Human OAS L has an inactive OAS domain (Marie I & Hovanessian A, 1992) with two additional domains of ubiquitin-like sequence (Hartmann R & Olsen H, et al 1998 and Hovanessian A & Justesen J, 2007). Even though human OAS L is inactive, when dsRNA binds, its activity is regulated through a cytosolic pattern recognition receptor, an essential molecule for the recognition of infected viral cells, unlike the other genes which are RNase L dependent (Alison M. Kell et Michael G, 2015).

OAS1 is the primary gene that encodes for the OAS1 protein. It consists of around 6 primary exons spanning 12,942 bp, there are however two additional exons present; one preceding the first primary exon and the one that is about 15 kbp away from exon 6. With the inclusion of both exons, the OAS1 gene spans 32,257 bp (Figure 1).



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Figure 1 The OAS1 gene consists of six primary exons, which translate into protein, with two additional exons on either side of the primary defined region of the gene. In total the whole gene spans around 32,257 bp. The primary exons are well documented and have been thoroughly studied. The other two exons have not been as well studied. These two encode for RNA transcripts. OAS1 gene is illustrated with the location of each exon with the promoter region location as well as the downstream end region location. The promoter region of this gene is found in the location chr12:112906758-112907061, within Exon 1b.

The OAS protein serves several purposes in addition to starting the RNase L pathway. For instance, studies on OAS1 have demonstrated that it possesses antiviral characteristics and may be secreted extracellularly and taken up by cells (Kristiansen H, Scherer C et al, 2010). OAS1 and OAS3 were found to suppress the expression of chemokine and interferon-sensitive genes in macrophages (Lee, W. bin & Choi et al., 2019). More research has shown that OAS1 downregulates the proinflammatory cytokine response of interferon-gamma-stimulated microglial cells (Magusali et al., 2021a).

These studies have revealed that the OAS locus has a broader function in the control of the immune system and in reducing inflammation. The neuro-inflammation process, which is known to contribute to both AD pathogenesis and the neurological symptoms of COVID-19, may be inhibited in part by this regulatory function. As a result, genetically rare variants may result in disorders worsening. OAS1 (chr12:112911065; GRCh38.p13) contains an uncommon genetic variation called Rs1131454, where the allele, guanine (GG), is replaced with the risk allele, adenine (AA), that has been shown to lower levels of functional OAS enzyme (Magusali et al., 2021b). The diminished regulation of the pro-inflammatory microglial response and the altered expression and activity of the OAS enzyme may be the common mechanism linking the effects of polymorphism in the OAS locus. Resulting in a higher likelihood of developing both LOAD and more severe symptoms from SARS-CoV-2 infections if the risk allele is present.

There are three types of interferons (IFNs): type 1 (which includes IFN alpha and IFN beta), type 2 (which includes IFN-gamma), and type 3. (IFN-lambda). In the presence of viruses like SARS-CoV 2, interferon signalling is upregulated by an amplification loop, which limits viral propagation by inducing a range of molecular effectors to suppress viral nucleic acid production, viral RNA digestion, transcription blockade, and protein function modification. Recent studies have linked Alzheimer's disease's neuroinflammation and synapse loss to the microglial interferon response. Uncertainty exists regarding the pathophysiology of Alzheimer's disease and the role of interferon signalling and antiviral immune response pathways in microglia.

1.5 Study Aims

The overall aim of this research is to identify variants at the OAS1 locus in 12 individuals. One group of four are confirmed AD individuals and homozygous for the OAS1 risk allele (AA), a control group with the protective allele (GG) and finally a control group with the risk allele (AA). The functional coding regions of the OAS1 gene have been studied previously but studying the non-coding regions

(introns) of this gene will give a more precise picture of genetic variation at this locus. If new SNPs are discovered this data can further our understanding and contribute towards potential linkage between SNPs associated with LOAD.

2. Methods

2.1 Study Design

A preliminary experimental research design is used to map variation in this region. This design is the most cost-effective approach for this study and is used as an attempt to observe and document potential new variants within the experimental group in conjunction with the controlled groups with and without the risk allele. The age demographic of all the subjects was within a similar age range and all >65, as the defined age for LOAD, providing a snapshot into a specific age range and the causalities that may affect LOAD (see Table 2 for demographics). The subject samples used were taken from a prior study (Magusali et al., 2021b).

12 DNA samples were obtained from the Alzheimer's Research UK (ARUK) DNA bank cohort and partitioned into 3 groups. 4 individual samples were homozygous AD with the risk allele in the OAS1 gene. 4 individual samples were control homozygote for the protective allele in the OAS1 gene and the last 4 individual samples were control homozygotes for the risk allele in the OAS1 gene. The risk samples have been genotyped for rs1131454 prior to the study and have been identified as risk carriers.

Table 1-Sample groups categorised in accordance with the ARUK DNA bank identification code.

Sample Groups		
1	2	3
AD samples homozygous for risk allele at SNP rs1131454 (AA)	Control samples homozygous for non-risk allele at SNP rs1131454 (GG)	Control samples homozygous for risk allele at SNP rs1131454 (AA)
LE319 – (7)	LE006 – (2)	LE073 – (1)
LE331 – (8)	LE041 – (3)	LE050 – (4)
LE402 – (9)	LE014 – (11)	LE075 – (5)
LE332 – (10)	LE016 – (12)	LE074 – (6)

Identification numbers of patient samples were obtained from the Nottingham ARUK DNA Bank with the corresponding individual groups. Homozygote AD samples for the risk alleles will be referred to as 1, Homozygote control samples for the non-risk allele will be referred to as group 2 and group 3 will be referring to the homozygote control samples for the risk allele.

Table 2- Demographic of samples carried out on the genotyping of SNP rs1131454 within the OAS1 gene from the ARUK DNA bank.

ARUK Ref No	Sex	Age	Group
LE073	Female	77	3
LE006	Male	68	2
LE041	Female	67	2
LE050	Male	72	3
LE075	Male	75	3
LE074	Female	76	3
LE319	Male	77	1
LE331	Female	78	1
LE402	Female	78	1
LE332	Female	79	1
LE014	Female	74	2
LE016	Male	67	2

Each sample was labelled with a number displayed next to the identification number given by ARUK, seen in Table 1. These numbers will be used to identify the samples as opposed to the ARUK ref No.

2.2 Primer design

Forward and reverse primers were designed in consideration of the gene fragment size. These primers were designed using an online tool called Primer3Plus (<https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>).

Several factors had to be considered when designing these primers, the first of which was the DNA fragment size. The final products would involve fragments that included 1a,1b, 2,3,4,5 and 6 exons. The forward and reverse primer pair had to sequence the first 15,000 bp of OAS1 (using Human Genome Assembly GRCh38.p14), with an inclusion of around 1000 bases upstream and downstream of the wanted fragment (see Table 3). Annealing temperature, binding site specificity and the presence of SNPs in the Primer sequence had to be considered. The specificity of each primer combination was calculated prior to using In Silico Polymerisation Chain Reaction (PCR) from the UCSC database. Primers were designed using the online tool Primer3Plus. Once designed, they were manufactured by Eurofins-Scientific.

Table 3- The annealing temperature and the product sizes that were expected from each primer combination trials for the OAS1 gene.

Trials	Forward Primer	Reverse Primer	Size Product	Annealing Tm (C)	Successful
1	OAS1Ex1aF	OAS1Ex6R	14,557	59	Y
	OAS1Ex6F	OAS1Ex7R	13,920	-	N
2	OAS1Ex1aF	OAS1a3R	8,130	60	Y
	OAS1Ex46F	OAS1Ex6R	7,683	66	Y

“Y” represents a successful trial, where the amplicon bands were distinct, and within the correct amplicon size. “N” represents an unsuccessful trial, where the

amplicon bands were unspecific. The first trial was unsuccessful. The subsequent trial, with a smaller product size, was successful.

Table 4 - The split primer nucleotide combination used for the Long Range PCR process of the OAS1 gene.

A		B	
Forward Primer OAS1Ex1aF	Reverse Primer OAS1a3R	Forward Primer OAS1Ex46F	Reverse Primer OAS1Ex6R
GTGCAAATTAAG GGCAGGTT	TTCTCTCTCTGTG ATCAGTGG	GGAGGAcaTGGG TGCTACTT	ACCTTGCACTCA CAGCACAT

These primer combinations were split in two; section A and section B.

2.3 Long-Range PCR

Long-range PCR would ideally be the best option to amplify DNA fragments of this size. Optimisation of annealing temperatures was carried out using general use DNA in preliminary PCR testing. An annealing temperature gradient of 58-63°C was first performed for each set of primers, and the products were then analysed via gel electrophoresis.

Table 5- The final concentrations and volumes of the reagents used for PCR of OAS1.

Reagents	Volumes added (µl)	Final Concentrations Used
H2O	152	-
5 X Buffer Platinum SuperFi II	48	1X
dNTPs	24	200µM
Forward Primer sequence	2.4	0.5 pmol
Reverse Primer sequence	2.4	0.5 pmol

Platinum SuperFi II DNA Polymerase	3.2	1.25 U/ μ l
DNA	8	20ng

Calculations for 8 products plus a blank of 30 μ l with excess to assure sufficient reagent mixture for PCR. The buffer solution and the DNA polymerase were obtained from the Invitrogen product line as “Platinum™ SuperFi II PCR master mix”. A reaction without a DNA template (blank) was included for each experiment, to ensure that there is no contamination.

Each PCR reaction contains a 30 μ l mix of reagent, comprised of 29 μ l of Master mix with the addition of 1 μ l of DNA material, separately.

Reactions were placed in a Verity 96 well (Applied Biosciences) thermal cycler with an initial denaturation phase temperature of 98°C for 30 sec followed by the secondary denaturation stage at 98°C for 10 seconds. During stage 2, annealing at the optimum temperature, depending on the primer combination, for 10 sec and an extension period at 72°C for 7 min 30 seconds. Stage 2 goes through this cycle 35 times. Finally, during stage 3 the DNA extension phase was operated for 5 min at 72 °C. The final PCR products were left suspended at 10°C when complete.

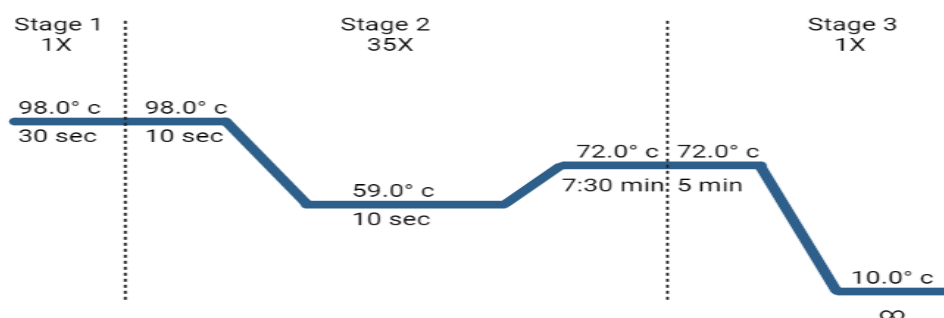


Figure 2 A thermal profile for PCR, showing the initial denaturation stage at 98°C, at 35 cycles with the inclusion of the denaturing at 98°C. Annealing took place at 59 °C with the corresponding duration for each cycle calculated in accordance to the Invitrogen PCR kit. The extension period is set at 72°C for 7.30 min for stage 2 and 5 min during stage 3.

2.4 Gel electrophoresis

PCR products were subjected to electrophoresis on a 0.6% agarose gel to confirm amplification. The gel was prepared by adding 0.60g of agarose to 100ml of 1X tris-Acetate-EDTA (TAE) buffer. The mixture was heated until the agarose was completely dissolved followed by cooling. 10µl of Ethidium Bromide with a concentration of 1µg/ml, was added to enable visualisation of DNA. The solution was then poured into a mould with well combs and allowed to set. After the hardening of the gel, it was submerged into TAE solution in a buffer tank. The well combs were subsequently removed carefully, to allow the loading of each PCR product. Each well contained 10µl from a combination of 5µl of PCR product mixed with 2µl of loading dye and 5µl of nuclease-free H₂O. A voltage of 40V for approximately 2 hours was applied to the gel, to determine the expected size of each DNA fragment.

Using a UV camera “AlphaDigiDoc software” the Ethidium Bromide fluorescent image of the gel was captured. Each amplicon was then compared to a 20kb ladder to determine whether they were the desired DNA fragment corresponding with the correct fragment length. The brightest amplicon corresponding to the temperature gradient with the least primer dimer formation observed, was the chosen condition for each subsequent PCR.

2.5 PCR purification

QIAquick PCR purification kit (QIAGEN) was used to eliminate extra primers, nucleotides, enzyme salts, and other undesired contaminants from PCR results. At first QIAquick columns were used to collect DNA fragments onto its membrane. One volume of PCR product was mixed with five volumes of Buffer PB. After mixing, a purple colour indicated an incorrect pH level, and 10µl of 3M sodium acetate pH 5 was added, turning the colour yellow. This mixture was applied to the QIAquick column and centrifuged at 13000rpm for 1 minute. Flow-through was discarded and 750µl of Buffer PE was added to each QIAquick column. These

columns were centrifuged for 1 minute and flow-through was removed. The columns were then centrifuged again for 1 minute at 13000rpm to remove any residual PE buffer.

Clean 1.5ml tube was used to contain the columns. 20µl of elution buffer (EB) was added to the centre of the membrane followed by 5 minutes incubation at room temperature. Columns were centrifuged for 1 minute at 13000 rpm to extract the purified PCR products.

To elute the DNA from the filter, 20µl of Buffer EB was added to the centre of the membrane and left to stand for 5 minutes. The column was placed in a clean tube and centrifuged again, allowing purified DNA to be collected.

A nanodrop Fluorometer was used to gauge the DNA concentration in each PCR product. Purified PCR product was combined with 20µl of working solution (Quant-iT™ dsDNA BR reagent in Quant-iT™ dsDNA BR buffer with a proportion of 1:200), which was then incubated for 5 min. 2µl of each purified sample was placed on the nanodrop to be analysed for fluorescence.

2.6 Sequencing

Next-generation sequencing (NGS) and Oxford Nanopore Technology (Deepseq) was used to identify the DNA sequence. Using the Qubit 4 Fluorometer and the Qubit 1X dsDNA HS Assay Kit from Thermo Fisher Scientific, amplicons were measured (Thermo Fisher Scientific; Q33231). The Native Barcoding genomic DNA procedure (Oxford Nanopore Technologies; Version: NBE 9065 v109 revS 14Aug2019) was used to create barcoded sequencing libraries for each amplicon pool using an equal number of the two amplicons from each sample (amplicon A and amplicon B). The Native Barcoding Expansion 01-12 kit and the Ligation Sequencing Kit (SQK-LSK109, Oxford Nanopore Technologies) were utilised in this protocol (Oxford Nanopore Technologies; EXP-NBD104). Barcoded libraries were pooled in equimolar amounts for the final sequencing-adaptor ligation. AMPure XP beads were used for every purification process (Beckman Coulter;

A63882). The final barcoded library pool was performed on the GridION X5 mk1 using a MinION flow cell (Oxford Nanopore Technologies; FLO-MIN106 R9.4.1).

2.7 Base-calling

Base-calling was performed on the GridION server using a high accuracy model. Reads were mapped to the 15.4kb reference sequence of the OAS1 gene using minimap2 (v2.22-r1101). Variants were called using Clair3(V0.1-r11) applying r941_prom_sup_g5014 model with phasing WhatsHap and Long Phase enabled. All other parameters were set to default.

3. Results

3.1 Gel electrophoresis

Using general-use DNA, the optimum temperature for both primers was successfully identified using gel electrophoresis. 60°C was chosen as the optimum temperature to be used for PCR involving the primers: OAS1aF - OAS1-3R (as seen in Figure 3(a)). 66°C shows the ideal condition for PCR amplification with the primer combination OAS1-46F to OAS1-6R (See Figure 3 (b) and 3(c)). The amplicon size seen for both primer combinations is within the expected base pair length range, and this is represented for all 12 samples in Figure 4.

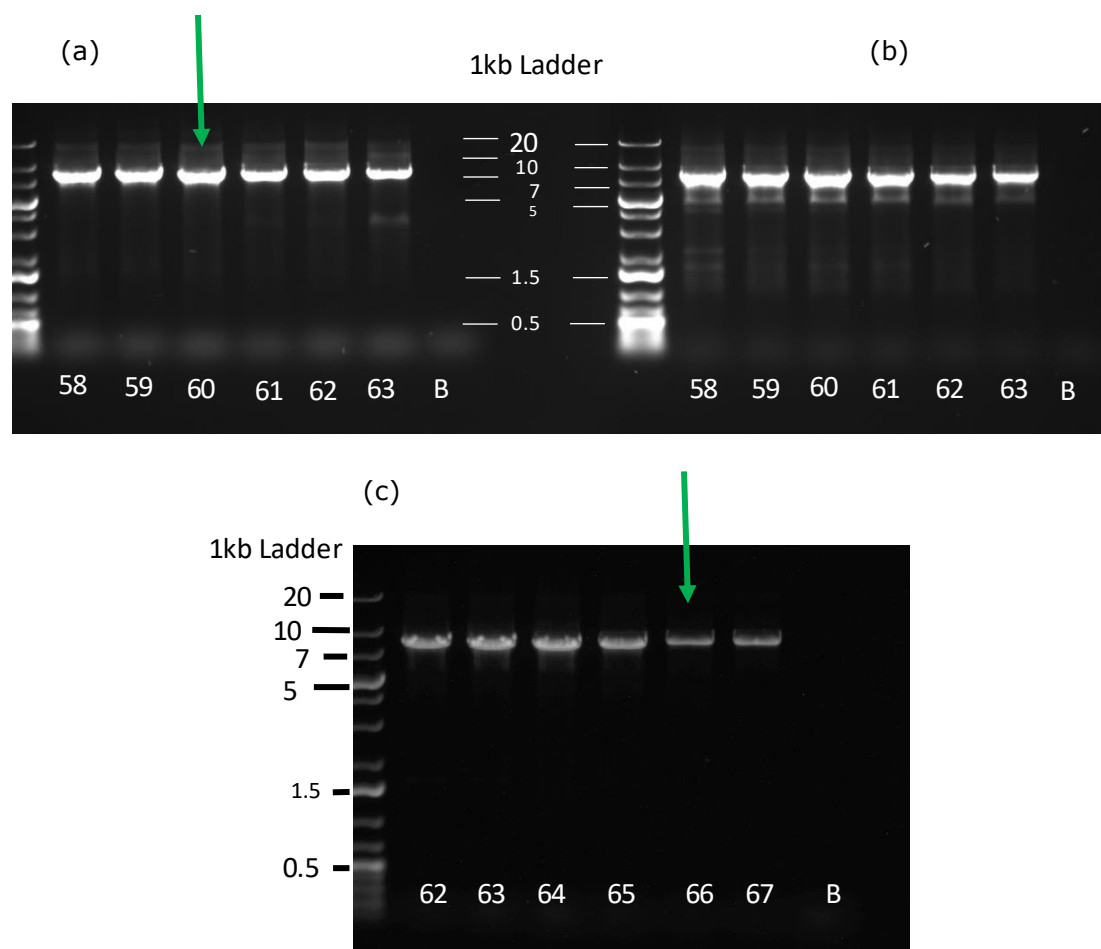


Figure 3 - Gel electrophoresis of General use DNA with primers combination: (a) OAS1aF to OAS1-3R, (b) and (c) OAS1-46F to OAS1-6R, at a temperature gradient of 58°C -63°C and 62°C -67°C respectively, on a 1kb Ladder (size ladder in the middle of (a) and (b), and to the side of (c)). The arrows show the optimal temperature used for the consequent PCR analysis. Amplicon formation was seen from 58°C -63°C with the correct band length of over 7kbp on (a). The amplicon seen at 63°C has a smaller size band appearing, however, they are not as prevalent as the main amplicon observed. At 60°C -62°C, the amplicon seems to be similar in intensity and size. (b) shows numerous band formations below the desired amplicon. Past 61, the formation of undesired amplicon size reduced. (c) shows the same part of the gene with the same primer combination as (b) with a higher temperature gradient. 62-65°C have faint smears below the amplicon. “B” refers to the blank, the negative control, and is seen to have no bands, confirming that no contaminants were present in the PCR mixture.

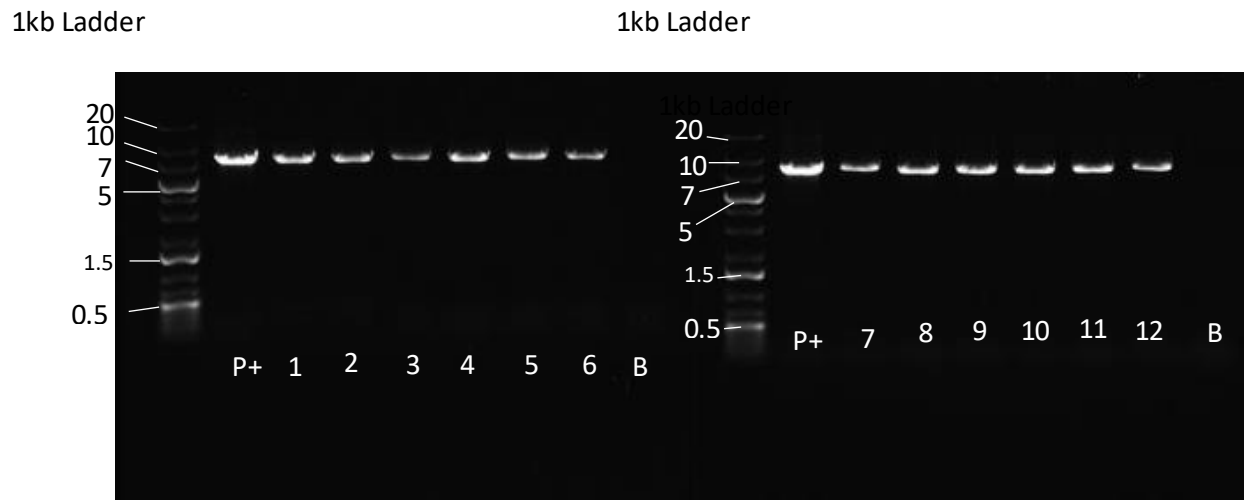


Figure 4 - Gel electrophoresis of 12 PCR samples, labelled at the bottom, with the addition of the positive control containing the first fragment of the gene (Primer combination: OAS1aF and OAS1-3R), with a 1kb ladder on the left hand side of the gel. All 12 amplicons are seen and positioned correctly on the DNA ladder, with the far-right column indicating a blank negative control. Amplicons show a size of just under 10k bp in length. Amplicons for OAS1-46F to OAS1-6R was also seen with similar bands (See Supplementary info).

3.2 PCR Product Purification

The quantity of DNA for each PCR product was determined by spectrophotometry. The concentration for each purified sample is presented below (See table 5).

Table 5 - The accumulated concentration of DNA from each purified DNA samples obtained from the PCR products of the OAS1 locus.

Sample Number	The concentration of DNA (ng/μl)			
	A	A - repeat	B	B - repeat
1	24.0	38.8	18.1	15.3
2	30.3	29.4	21.7	29.8
3	31.0	32.1	15.5	20.2
4	34.1	33.7	23.1	24.5
5	32.3	35.4	22.9	22.3
6	22.5	25.9	21.9	21.3
7	23.7	24.4	16.9	16.4
8	40.4	30.9	21.3	20.0
9	34.5	35.3	17.0	16.4
10	33.2	36.0	34.5	35.1
11	26.8	26.1	25.3	23.0
12	30.6	30.7	18.7	27.7

Both Amplicons A (OAS1(1a-3R)) and B (OAS1(46F-6R)) are represented. The concentration of DNA for all the samples was obtained with the aim of around 20ng/μl overall, to ensure maximum quality reads in sequencing.

3.3 Sequencing Analysis Summary

Table 6- The output from the alignment of each sample, referred to as barcode followed by sample number, to the reference OAS1 sequence (GRCh 38- hg38).

Sample	#r name	start pos	End pos	Num reads	Cov bases	Coverage	Mean depth	Mean baseq	Mean mapq
Barcode 01	OAS1	1	15,400	115,484	14,931	96.95	48,427	22.4	59.6
Barcode 02	OAS1	1	15,400	108,898	14,920	96.88	46,180	22.4	59.7
Barcode 03	OAS1	1	15,400	87,827	14,924	96.91	37,194	22.4	59.6
Barcode 04	OAS1	1	15,400	125,481	15,205	98.73	52,529	22.4	59.6
Barcode 05	OAS1	1	15,400	115,240	15,233	98.92	48,288	22.4	59.6
Barcode 06	OAS1	1	15,400	113,627	14,926	96.92	48,135	22.4	59.6
Barcode 07	OAS1	1	15,400	97,241	14,935	96.98	40,944	22.4	59.7
Barcode 08	OAS1	1	15,400	101,771	14,924	96.91	42,868	22.4	59.6
Barcode 09	OAS1	1	15,400	99,517	14,937	96.99	41,360	22.4	59.5
Barcode 10	OAS1	1	15,400	110,220	14,899	96.75	45,832	22.4	59.7
Barcode 11	OAS1	1	15,400	118,459	14,933	96.97	49,678	22.4	59.8
Barcode 12	OAS1	1	15,400	103,594	14,953	97.10	44,143	22.4	59.7

Every fragment covered the majority of the reference sequence (see appendix) for the OAS1 gene, as seen in Table 6. The "startpos" and "endpos" refer to the positions of the bases that were used from the reference. The total number of reads for each sample is referred to in the "numreads" column. The "coverage" refers to

the unique reads that include the given nucleotides from the reference sequence as a percentile. Every sample analyzed and sequenced has a coverage of over 95%. The mean depth refers to the sum of mapped reads depth observed divided by the known number of bases from the entire reference sequence. This is a metric to indicate the number of reads on average that are likely to be aligned correctly at a given reference base position. The “mean base q” refers to the average qscore of bases from each mapped reads. “Mean map Q” refers to the average quality of mapping from mapped reads.

To examine variations found from each sample, a Variant Calling Format (VCF) file was generated, from the sequencing data (See supplementary section). This VCF file contains the location of the SNP in relation to the reference sequence, the Qscore, the reference allele with the alternate allele that appears in the sample sequence and states whether the variation is heterozygous or homozygous. Each SNP was post-call filtered, to identify the “Quality”. Using the VCF file, each SNP was categorized to the data that follows.

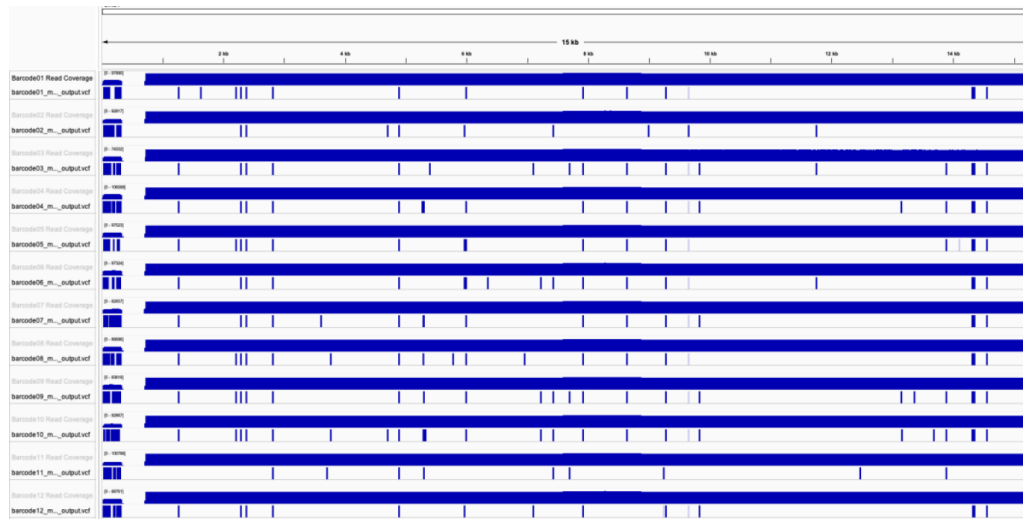


Figure 5 - A visualization of read depth, coverage and variants for each sample using IGV. The OAS1 sequence against which the reads were mapped is shown at the top of the figure. Tracks labelled "read Coverage" show read depth. Variants are shown as vertical lines in track labelled barcodeXX_merged_output.vcf.

3.4 Qscore of SNPs

Table 7 - Frequency of SNPs found in each sample with an emphasis on the Qscore calculated and categorised for each SNP.

Qscore Sample	<10 (%)	10-14.99 (%)	15 -19.99 (%)	20 -24.99 (%)	25 -29.99 (%)	>30 (%)
1	14	0	0	0	1	4
2	23	0	0	0	0	0
3	19	2	0	0	0	1
4	16	5	0	1	2	4
5	8	1	1	0	0	3
6	13	4	0	0	0	5
7	24	5	0	0	0	3
8	18	3	0	0	2	6
9	18	6	0	1	1	6
10	20	0	0	0	2	5
11	14	0	0	0	1	0
12	19	2	1	0	0	0

Most of the SNPs found below 10% are ignored and are probably false positives.

Qscore refers to the quality of the sequencing of a given base. $Q = -10\log_{10}(e)$ is the equation used to calculate Qscore, and “e” is the estimated probability of a specific base call being incorrect. The higher the Qscore, the smaller the probability of an error occurring for a given base. A lower Q score can result in inaccurate conclusions as these reads are more likely to be errors and therefore unusable. A QScore of 30% and above is considered the benchmark for quality reads in NGS. Many SNPs found in each sample are below 10% and therefore unusable. Short read sequences have a higher accuracy in quality checking (Malmberg et al., 2019). Initially 30% was considered, however this study uses long read sequences, adhering to the 30% benchmark could discredit SNP findings that are below this level, especially since NGS is a fairly new technique that requires refining. A select few SNPs between 15% and 30% were taken for consideration, as many of the SNPs found within these categories were identified within the dbSNP database. Many of

these SNPs, corresponding to the over 15%, were also found in risk allele sample groups with and without AD at a higher Qscore. Only a select few SNPs were seen in the control group without the risk allele. The average Qscore for this dataset is 17.8%, with an average number of reads over 108,000.

3.5 Identified SNPs

Table 8 - Identified SNPs present in their respective groups with the ancestral and alternate base callings from each location seen in the samples.

SNP RefLoc	Location (Ch12:)	Mutation Type	Rs No	Ancestral Allele	Alternate Allele	Group
43	112905116	-	-	T	C	2
2378	112907451	Substitution	rs10744785	C	G	1,3
2821	112907894	Substitution	rs4766662	A	C	1,2,3
5271	112910344	Substitution	rs138617415	G	A	3
5783	112910856	Substitution	rs34137742	C	T	1
5992	112911065	Substitution	rs1131454	G	A	1,3
6955	112912028	Substitution	rs55676265	A	G	1
7227	112912300	Substitution	rs75344264	A	G	1, 3
8642	112913715	Substitution	rs2285934	T	G	1,3
9281	112914354	Substitution	rs4767023	T	C	1
9830	112914903	Substitution	rs45495393	C	A	1,3
12479	112917552	Substitution	rs148741042	T	G	2
13157	112918230	Substitution	rs60623134	A	G	1,3
14315	112919388	Substitution	rs10774671	G	A	1
14331	112919404	Substitution	rs1131476	G	A	1
14359	112919432	Substitution	rs1051042	G	C	1,3
15228	112920301	Substitution	rs7135577	A	G	1,3

Group 1 refers to homozygote AD samples with the risk allele, Group 2 refers to homozygote control samples with the non-risk allele and Group 3 refers to homozygote control samples with the risk allele. All these SNPs are above the 15% Qscore threshold. “SNP RefLoc” refers to the position of the relevant SNP, by the reference sequence used (see Appendix). The location of the allele was identified using the GRCH38 reference assembly.

Table 9 - SNPs present in each of the independent sample groups categorised by the overall calculated Qscore range.

a)	Group		
SNPs over 30% Qscore	1	2	3
rs1131454	X		
rs10744785	X		X
rs4766662	X	X	X
rs1051042	X		X
rs7135577	X		X
rs2285934	X		X
rs60623134	X		
rs75344264			X
rs10774671	X		
rs4767023	X		
rs1131476	X		

b)	Group		
SNPs 25%-29.99% Qscore	1	2	3
rs1131454			X
rs138617415			X
rs60623134			X
rs34137742	X		
rs55676265	X		
rs75344264	X		
rs45495393	X		
rs148741042		X	

c)	Group		
SNPs 20-24.99% Qscore	1	2	3
rs45495393	X		X

d)	Group		
SNPs 15-19.99% Qscore	1	2	3
rs4766662			X
Ch12:112905116		X	

Group 1 refers to homozygote AD samples with the risk allele, Group 2 refers to homozygote control samples with the non-risk allele and Group 3 refers to homozygote control samples with the risk allele. SNPs with the corresponding rs number are documented SNPs found on dbSNP. “Ch12:112905116” is the location of a SNP, using the hgCh38 genome Assembly. A) represents the SNPs with over 30% Qscores. B) represents the SNPs that had between 25% and 29.99%. C) represents known SNPs with Qscores between 20% and 24.99%. D) represents SNPs with Qscores of between 15% and 19.99%.

Every SNP identified over 30% Qscore (Table 9 (a)) are confirmed documented SNPs in dbSNP that are present at the OAS1 gene locus. Certain SNPs such as rs45495393 appeared on numerous occasions from several sample sequences, with different Qscores. Rs45495393 was seen in sample 4 (23.34%), 9 (24.91%) and 10 (26.23%) (see supplementary data). A select few SNPs such as rs47666622 were also found in the control group without the risk allele. SNPs that were below 15% were ignored as there is 1 in 10 chance that these reads are incorrect.

Deepseq sequenced the samples to produce over 1 million readings. On average 108k reads were mapped to the reference covering 97.25% of the reference sequence at a mean depth of 45,465. Variant calling identified between 37 and 57 variants per sample. Some of which were heterozygotes in nature.

RefLoc 2821– (Rs4766662)

Rs4766662, a homozygote SNP was identified with a Qscore of above 30% in only one sample in AD risk and the control non-risk group compared to 3 samples from the control risk group. In contrast, one sample from both the AD risk and the control risk group was seen with a Qscore of 14.84% and 16.04% respectively, these carried the heterozygote allele. This variant is located before exon 2.

RefLoc 2378 – (rs10744785)

A transversion substitution SNP was seen in three samples from the control risk group; 1, 6 and 10 with Qscore of 34.23%, 30.81% and 31.82% respectively. This SNP is located after exon 1 in the intronic region of OAS1, at position Ch12: 112907451. After a further inspection on dbSNP, this variant is categorized as rs10744785. This was seen in three individuals with homozygous (GG) instead of heterozygous (CG), where the Cytosine is substituted with a Guanine. With a sample size of 8318 in the literature, the frequency of this mutated allele appearing is 0.66 globally, the majority appearing in the European populace (Accessed statistics from National Center for Biotechnology Information, NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs10744785>, in July 2022). The high Qscore seen in all samples can justify the positive presence of this allele.

RefLoc 5271-rs138617415

Only one control risk sample was seen to have this heterozygous variant with Q 27.35% where the A allele replaces the G allele. This SNP is located on Chr12:112910344, after exon 2 on the second intronic region of OAS1. Due to the lack of reports on this mutation and global frequency (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs138617415>, in July 2022), it is possible that this specific mutation in sample 4 is a false positive, or it could be a rare variant.

RefLoc 5783- rs34137742

In the same vein as rs138617415, this SNP only appears in one AD risk sample as a heterozygous variant with a Qscore of 28.23%. This SNP is identified as rs34137742, replaces C with a T allele and is located on Chr12:112910856, close to the acceptor splice site of Exon 3. Unlike rs138617415, rs34137742 is more prominent with a frequency of 0.10 globally (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs34137742>, in July 2022).

RefLoc 6955- rs55676265

This SNP was also seen in the same AD risk sample as rs34137742, with a Qscore of 29.25%. It is a heterozygous variant (GT) positioned on chr12:112912028 replacing the A allele with a G, in the third intronic region of OAS1. Using the reference and position, this SNP is identified to be rs55676265, with a frequency appearance of 0.20 worldwide (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs55676265>, in July 2022).

RefLoc 7227- Rs75344264

Unlike the previous SNP, this particular SNP appears as both a homozygote and a heterozygote. SNP Rs75344264 is found on chr12:112912300 in the third intron of OAS1 and was seen only seen in one sample from the control risk group as opposed to the two samples found in the AD risk group. Both AD risk samples had the

heterozygous variant (GT) of the SNP whilst the control risk sample had the homozygous variant with a higher Qscore of 33.37% compared to the heterozygous variant of 27.57%, and 27.55% respectively.

RefLoc 8642- rs2285934

SNP rs2285934 has a homozygous allele G instead of T in 2 samples from both the control and AD risk groups. The control risk samples had a higher Qscore reading compared to the AD risk samples with 40.21%, 39.69%, 38.77% and 38.66% respectively. This is a variant that seems to appear far more frequently than its ancestral counterpart with 0.64 for the G compared to T with 0.35, globally (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs2285934>, in July 2022). It is located on chr12:112913715 on the third intron region of OAS1.

RefLoc 9281- rs4767023

Also known as rs4767023 this variant is homozygous with T replacing C in only one sample from the AD risk group, with a Qscore of 32.58%. This is located on chr12:112914354, in the third intron region of OAS1. This variant, similar to rs2285934, has been cited more frequently than its ancestral counterpart.

RefLoc 9830 - rs45495393

2 samples from the AD risk group had the heterozygous variation of this SNP with Qscore of 24.91% and 26.93%, whilst only one sample from the control risk group, with a lower Qscore of 23.94%, was seen with this SNP. SNP rs45495393 replaces the C allele with the A allele, another variant present in intron 3 like rs112912028, however, it is closer to the acceptor splice site of exon 4. According to the ALFA project, this SNP has a frequency of 0.10 for the A allele (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs45495393>, in July 2022).

RefLoc 12479- rs148741042

Heterozygous variant rs148741042 was only seen in one sample from the control non-risk group with a Qscore of 26.61%. This SNP changes the T allele to a G. Due to its location in the fifth exon of OAS1; it will affect translation and is a missense variant. It is however not a frequently observed variant, with a global frequency of 0.0005 according to the ALFA project (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs148741042>, in July 2022). TGT is the codon that includes the ancestral allele, coding for the amino acid Cysteine. With the inclusion of the change, this becomes non-synonymous for Tryptophan TGG.

RefLoc 13157- Rs60623134

Rs60623134 is a heterozygous variant seen in one control risk sample with a lower Qscore of 28.36% compared to one other sample from the AD risk group with a Qscore of 30.06%. It replaces A with G and is located in the fifth intron of OAS1, and therefore will not have an effect on protein translation. According to the ALFA project, this SNP has a frequency of 0.08 globally (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs60623134>, in July 2022).

Ref Loc 14315- Rs10774671

Rs10774671 is a homozygous variant that replaces G with A and was only seen in all four of the AD risk samples with 31.38%, 36.43%, 33.15%, and 35.07% Qscores. It was only seen in the AD group with the risk allele, this variant therefore, may play a significant role. This particular variant is on the Acceptor splice site of the sixth exon (chr12:112919388).

Ref Loc 14331- Rs1131476

Rs1131476 was only seen in one AD risk sample with a Qscore of 34.44%. This SNP replaces G with A. The frequency at which it appears in global statistics taken from the ALFA project with 0.65 (Accessed statistics from NCBI, <https://www.ncbi.nlm.nih.gov/snp/rs1131476>, in July 2022). This is a reported exonic mutation in the sixth exon (chr12:112919404) of OAS1.

RefLoc 14359- Rs1051042

Rs1051042 was seen in all samples from the risk group with and without AD. This SNP involves homozygous alleles of CC instead of GC. All 8 separate instances of this SNP were above 30% in Qscore (see Supplementary section). It is located on the sixth exon, conforming to the idea that it is a missense variant.

RefLoc 15228- rs7135577

This SNP also appears in all 8 samples in the risk group with and without AD. This variant similar to rs1051042 is a homozygous variant with G instead of A. Also known as rs7135577, it is an intron variant located on chr12:112920301 past exon 6 in the sixth intron of OAS1. Similar to rs1051042 every sample from the groups containing the risk allele had this variant with Qscore over 30%.

Pos 43 – (SNP unknown)

One SNP at the RefLoc position 43 was seen with a Qscore of 16.64% similar to SNP rs4766662. This SNP was only seen in one sample from the non-risk control group, with a Homozygous CC allele, instead of the reference TC. This variant does not match any known dbSNPs at the aligned location and could be a potential new variant albeit the Qscore is low.

Most of the relevant identified SNPs are located in the latter part of the OAS1 gene. Many of these SNPs are intronic in nature and only appear in one sample. The positive control risk allele rs1131454 was only seen in two samples (see tables 8 and 9) with a Qscore of 29.63% for sample 1 and 30.11% for sample 8. These are unlikely to be false positives. However 8 of the DNA samples, should have included a SNP presence for rs1131454. The remaining 6 samples were not base called for low-quality readings of the control SNP. The presence of this SNP was non-existent. These samples could have been chosen for this particular study incorrectly. NGS is a novel methodology, so there is a possibility that there could be errors in the sequencing.

4. Discussion

Sequence data obtained from Deepseq, verified the presence of numerous SNPs with a high degree of confidence. The control risk allele for AD was only present in 2 samples, the absence of the variant in the other samples means that this study lacks validity. SNPs: rs7135577, rs10774671, rs1051042, rs4766662, Rs10744785 and Rs2285934, were sequenced in numerous risk-allele associated individual samples with high-quality reads, proposing the idea that they may be relevant to understanding AD. However, SNPs that appear infrequently amongst the same group cohort, similar to Rs2285934, may suggest that they may not be relevant to AD. They may have different pathogenic effects or may be non-pathogenic, this will need to be investigated further.

4.1 Variants of Interest

SNP rs7135577

SNP rs7135577 is a variation found in 8 individuals, from the control risk allele group and the AD risk allele group. This SNP is an intronic variant in the 3'UTR and does not seem to have been reported for any clinical significance in ClinVar. There has only been one reported study with this SNP, suggesting an LD to rs10774671 (Lim et al., 2009), which was also seen in the same individuals from our sample set. This study can be used as further evidence for confirming the presence of a linkage between these two SNPs.

SNP rs10774671

As previously stated, this SNP has been seen to be in a haplotype with rs7135577 (GA), with a high LD (Lim et al., 2009). R^2 value of 1 was established (see supplementary section), between rs10774671 and rs7135577 in this study, suggesting a perfect LD. Due to the small sample size, this value cannot be used as

evidence for a linkage between the two SNPs however it is an interesting correlation. SNP rs10774671 is located on the splice acceptor site of exon 6, with a variation of A from the ancestral G allele. Variation A is the result of the p42 isoform of OAS1, where the splicing of the 3' terminal exons of OAS1 creates transcripts for other OAS1 isoforms, in this case, p42, whilst the G is the result of the p46 isoform. It was seen in only 4 of the individuals, all of whom are in the AD group with the risk allele.

This SNP has been studied for its direct implications for numerous viral conditions. This includes the West Nile Virus infection (WNV) (Lim et al., 2009), hepatitis B virus (HBV) infections (Liu et al., 2017), hepatitis C virus (Bader El Din et al., 2015) and tuberculosis (TB) (Wu et al., 2018). Through the observation of all these individual samples containing this SNP, it is the p42 variant that seems to be prominent. Whether this suggests a link between this and the risk allele and more importantly AD, cannot be assumed. The absence of the risk allele reading in the control risk samples and the risk samples also means that LD between these two SNPs cannot be validated. Using bioinformatics tools to calculate LD, these two SNPs did not compute with a significant r^2 value. This could mean that there is no LD or it could also mean that there weren't enough data to provide a significant r^2 value (see supplementary section). However, with a larger sample size, the LD of this SNP to the risk allele could be ascertained. Using LDlink, it is suggested that the r^2 value is around 0.72 in the European populace, with a lower r^2 value globally excluding Europe (Accessed data on Feb 2023 using LDlink API on R- <https://ldlink.nci.nih.gov/?tab=home>).

Further investigation is required for a potential robust LD between this SNP and the SNP rs6489867 (T) that increases the risk of SARS-CoV-2. The influence of this SNP on tuberculosis with the presence of rs10735079 and rs6489867 could be used to further understand the pathogenesis of SARS-Cov-2. A more recent study investigating the non-Neanderthal SNP rs10735079 (A) haplotype, reported to elevate SARS-CoV-2 risk, was seen to be in association with rs10774671- A

(Banday et al., 2021), invoking further thought that factors regulating OAS1 expression can be used as a way to combat SARS-CoV-2.

SNP rs1051042

Rs1051042 is a SNP found in 8 individual samples, all of which were in the AD risk group and the control risk group. This specific SNP is in the sixth exon and replaces the G ancestral allele with a C on the 5' end of the DNA. The data suggests that this SNP is a homozygote variant present in all the individual samples with a CC. This results in a missense variation, a nonsynonymous change from the GGA amino acid coding for glycine to CGA, Arginine. This change does not seem to have any clinical significance, however since this SNP seems to appear in both risk groups, it does warrant some further investigation, as there is not enough research done on this SNP in different populations. LD calculations suggest that this SNP may have very low LD or may be in linkage equilibrium, global statistics also suggest this with a r^2 value of 0.0007 (Accessed data on Feb 2023 using LDlink API on R- <https://ldlink.nci.nih.gov/?tab=home>).

A study investigating an association with OAS1 with prostate cancer found that this particular SNP along with SNPs rs1131454 and rs10774671 are in LD with rs2660, a SNP located in exon 7 of OAS1 (genotype AA) that was deemed significant for the increased risk of prostate cancer (Mandal et al., 2011). Only one study was conducted relating to this SNP. With a sample size of 214, the study has enough power to support the conclusion made. However further investigation needs to be conducted to study the proposed linkage between these SNPs, and the mechanisms that may cause these conditions within OAS1.

SNP rs4766662

Rs4766662 is an intronic SNP, on the sense strand, found in the 5 individuals, where it appears in 3 of the control samples with the risk allele, with the homozygote CC genotype and 1 control sample with the risk allele with heterozygote CT genotyping. The pathogenic relevance of AD is yet unknown. The two other samples that contained this variant were in the two other groups, with CT genotyping. Due to its presence, in a few samples, it is likely to be a benign variant. With a larger sample size investigating this SNP, its association could be investigated.

Samples 5 and 7 contained this SNP however the Qscore was below the preferred margin of 30%. Both Qscores were 16.04% and 14.84% respectively. These could be presented as errors in the sequencing, however, due to the multiple appearances of this SNP in other samples with multiple Qscore of over 30%, it is most likely to be a positive variant appearance.

Rs10744785

Rs10744785 represented an apparent substitution of C to G, seen in the sequences of samples 1,6 and 10. Due to the small sample size in this study, any association cannot be made, as this SNP seems to appear in both homozygote AD samples and the control samples with the risk allele. This SNP however seems to be associated with a significant increase in measles-specific humoral immune response (Haralambieva et al., 2011). This SNP does lead to a non-synonymous change to Arginine instead of Proline, however, it is yet unknown as to how it causes an increase in antibodies that neutralize measles. It also seems to be in an LD block with rs4766662 and rs7956880 both of which are in the first intron of OAS1 and rs2158390 which is found at the 5'UTR (Lim et al., 2009). This SNP could be an important genetic marker to identify certain pathogenic diseases. This study can be used to provide preliminary data to potentially support an association with rs4766662, however, only with a much bigger sample size, can this be verified.

Rs2285934

Rs2285934 is an intronic variant, that occurs after Exon 3. It replaces a T nucleotide with G. This variant was seen in four individuals, from the control group with the risk allele and the AD group with the risk allele, with the homozygote variant GG. Its function, if any, is largely unexplored, however, a study in 2017 suggests that a polymorphic change in this SNP is associated with the severity of liver disease in HIV patients. The presence of a heterozygote TA allele was seen to decrease the severity of Liver disease (García-Álvarez et al., 2017). This suggests this SNP plays a significant role in hepatic fibrosis progression. However, there does not seem to be any visible association between this SNP and AD in the literature. Despite this, the presence of this SNP in conjunction with the risk allele cannot be dismissed. Rs2285934 may form a haplotype with the risk allele and other alleles. This indicates that further research on this SNP's effects and its haplotypes will need to be conducted. This mutation may have an impact through its association with other alleles.

Several SNPs only seem to have been sequenced in one or a few individuals. This is in direct contrast to rs7135577 and rs10774671, which appear in all 8 individuals with the risk allele. rs55676265, rs34137742, rs45495393 and rs138617415 are unlikely to have any association with LOAD however this cannot be concluded as a definite evaluation without increasing the sample size of individuals with these SNPs and investigating the susceptibility of increased risk to LOAD. rs138617415 and rs148741042 are SNPs that are seen with extreme rarity, according to statistics provided by ALFA. This could either mean that they are artefacts or potential low-frequency rare variants. These low-frequency variants have rather weak phenotypic effects, to study them, a large cohort will need to be studied. In contrast, rs1131476 is an SNP that appears in a far greater frequency than its ancestral allele counterpart. rs1131476 (previously called rs3177979) is a missense SNP in the coding region of exon 6. Its current pathogenic effect is unknown, it may play a role in prostate cancer, as it is in LD with rs2660 (Mandal et al., 2011). It may be of interest to

investigate whether this potential LD also exists as a block within this sample set and with individuals with the risk allele.

SNP Position 43

This SNP, positioned on ch12:112905116, is the only potential novel SNP that has a Qscore between a band of 10% and 20%, appearing in sample 12 with 16.64%. There were multiple appearances of this SNP in other samples from the control group with the non-risk allele and the AD group with the risk allele, however, the QScores are below 10% and therefore deemed unreliable for analysis. This particular SNP appearing in sample 12 could likely be an error in the sequencing, as none of the other samples displayed this specific SNP. Despite the Qscore being 16.64%, and confirmed SNP rs4766662, also having Qscore below 20%, it could mean that the appearance of this SNP could also likely be positive. This needs to be investigated further with a greater sample size, to verify the likelihood of it being potentially novel.

4.2 Limitations

Producing amplicons of over 10kbp in length using Long Range PCR can be arduous, as several factors can inhibit a successful amplification. The first trial involved using a set of primers that were designed to amplify the OAS1 gene including the seventh exon. This would have resulted in the amplification of two PCR products around 17kbp totalling 33kbp in total, with subsequent full analysis of the OAS1 gene. This methodology was rejected due to the lack of specificity and reproducibility. After amplification, there were several undesired bands, compared to the increased specificity that was needed for the successful sequencing of PCR products. This lack of specificity may have been a result of using primers that may have had SNPs or may have bound themselves to completely different parts of the human genome resulting in several band formations.

To rectify this issue Dimethyl Sulfoxide (DMSO) and a range of different temperatures were tested using the first set of primers, all resulting in the absence of amplicons or smearing. DMSO binds to DNA at the cytosine residue and changes its conformation which makes the DNA more labile to denaturation. The annealing temperatures for this set of primers were increased from 52 degrees to 69 degrees. 72 degrees was the denaturation temperature and therefore, an annealing temperature higher than this could not be used.

dNTPs and other reagents used may have affected the PCR products, as freeze-thawing, the buffer solution and dNTPs cause gradual degradation as a consequence of exo/endonuclease activity. A different buffer mix provided by Qiagen was also utilized but produced similar results.

There were numerous complications when analysing each PCR product in 1% agarose gel. Larger molecules separate better when using lower-concentration gel. At 1% agarose concentration, the large DNA fragment could not separate, and the product was left in the well. At 0.75% the same effect was observed but to a lesser extent. 0.6% seems to have been the optimal concentration for this size fragment, as the gel's larger pores facilitate the separation of larger fragments. Making the gel 0.5% would increase the separation and further facilitate the observation of separate bands, to correctly judge the size of each amplicon.

After having sequenced the samples, several SNPs were found multiple times. COVID-19 SNP rs6489867 was never identified due to the size of each fragments that were used for PCR. The control risk allele rs1131454 was only seen in two samples, in their respective groups. Making any assumptions on LD between the risk allele and the SNPs found in these samples would be inaccurate. All these issues directly diminish the reliability of this study considerably. To reliably detect and amplify these alleles, real-time PCR Taqman could be used. This will eliminate the issue of using samples that may or may not have the risk allele, as well as give us a better understanding of whether the sequencing method was an issue. NGS is a novel sequencing method and does not have the accuracy of Sanger sequencing.

These potentially new SNPs that were seen at a lower Qscore may already have been found and documented under a different identity. Increasing the power of the study, by sequencing additional AD samples with and without the risk allele, would be a viable first step in identifying whether these SNPs are outliers or artefacts.

Rs1131454 and rs10774671, two identified risk alleles to have associations with the severity of COVID-19 and SARS-CoV-2, seem to be prominent in the European population. Studying a non-European cohort and investigating potential risk alleles would be a beneficiary step in identifying the risk of severe disease. This study lacked the statistical power as well as the additional functional variants present in non-European populations to correctly identify any potential associations with AD or COVID-19. Some of the control subjects may develop AD in the future, this could not be accounted for in this study. It is stated that age is a major factor in the development of LOAD, as the prevalence of LOAD in persons aged 65 to 69 is around 0.6%, increasing to 22.2% in individuals aged 90 and above (Lobo et al., 2000). Using different age range cohorts, from 80-90, in addition would greatly benefit this study.

Preliminary experimental studies are extremely unreliable since they cannot rule out hypotheses with any degree of confidence. To negate this for consequent studies, a candidate gene study, structured as a case-control approach could be a viable alternative, as this would explore this specific locus and it would provide enough data to investigate LD. This study neither confirms nor rejects the idea that these variants are associated with any viral infections or LOAD but has provided preliminary data that merits further investigation.

4.3 Future Implications

This study provides information for the existence of multiple SNPs in the OAS 1 gene whilst also shining a light on what could potentially be an association between the risk allele and these documented SNPs. Further studies, to confirm the existence of a link between these SNPs and the risk of developing LOAD, need to be conducted to better understand the pathogenesis of the disease. This would provide a focal point for future research involving the mechanism of OAS and neuroinflammation. OAS2 and 3 will also need to have both intronic and exonic data sequenced, this will confirm documented SNPs, whilst also introducing new SNPs and potential LD blocks that may be associated with LOAD. The lack of investigatory studies involving these SNPs and the development of AD in individuals means that further research needs to be conducted for associations between SNPs and the risk of attaining AD.

Whilst this study was not powerful enough to identify the LD of SNPs with the risk allele, there have been SNPs that seem to be consistent in appearing in AD individuals. A sample size of over 250 in each respective group would statistically provide enough power, to investigate a single SNP marker. With an increased sample-sized study, distinguishing new alleles that could have the same effect as the risk allele, may also give us a better understanding of the pathogenesis of LOAD and new associations to other alleles that could have an underlying alteration in the development of AD. However increasing the sample size to 250 and above, may come at an increased monetary cost, if NGS is to be utilised. Investigating recurring mutations of the OAS1 gene in the general population can also be beneficial as it may provide us with a better understanding of the factors involved in both AD and COVID-19.

OAS locus harbours numerous variants that play roles in viral infections such as WNV, TB and prostate cancer. OAS3 and OAS2 may be significant in the contribution towards developing severe disease. Two missense OAS3 variants were

reported to be especially potent in Europeans, independently causing an elevation in severe COVID-19 risk (Banday et al., 2021).

5. Conclusion

In conclusion, this study explored sequence data from the OAS1 gene excluding the intronic region after exon 6 and observed the existence of multiple known variations, reported on dbSNP and observed a potential SNP, at position chr12: 112905116 of OAS1, that needs to be further investigated. The results obtained from this study can be used as preliminary data for future studies. The link between OAS1 and AD needs to be considered further, especially the association between the risk allele and the aforementioned SNPs, and whether they are in a haplotype. The OAS locus itself may provide future project materials and information for clinical use to combat AD, WNV, TB and more recently, severe COVID-19.

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Supplementary

Table 10- VCF file content, showing every SNPs present in OAS1 from all 12 samples.

#CHROM	POS	Sample	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	barcode01			
OAS1	25	1	.	G	.	2.77	RefCall	F	GT:GQ:DP:AF	0/0	2	10	0.6
OAS1	33	1	.	C	.	8.82	RefCall	F	GT:GQ:DP:AF	0/0	8	18	0.6111
OAS1	34	1	.	T	.	7.71	RefCall	F	GT:GQ:DP:AF	0/0	7	18	0
OAS1	36	1	.	A	.	4.69	RefCall	F	GT:GQ:DP:AF	0/0	4	24	0.5833
OAS1	43	1	.	T	C	8.16	PASS	F	GT:GQ:DP:AF	1/1	8	39	0.7436
OAS1	45	1	.	C	T	9.34	PASS	F	GT:GQ:DP:AF	0/1	9	40	0.375
OAS1	46	1	.	G	A	9.41	PASS	F	GT:GQ:DP:AF	0/1	7	44	0.5909
OAS1	50	1	.	A	G	3.74	PASS	F	GT:GQ:DP:AF	0/1	3	44	0.3864
OAS1	81	1	.	A	G	6.5	PASS	F	GT:GQ:DP:AF	0/1	18	50114	0.7117
OAS1	88	1	.	G	A	7.05	PASS	F	GT:GQ:DP:AF	0/1	34	50043	0.9022
OAS1	125	1	.	T	.	7.18	RefCall	F	GT:GQ:DP:AF	0/0	9	39	0.359
OAS1	127	1	.	C	G	4.03	PASS	F	GT:GQ:DP:AF	1/1	25	50006	0.7269
OAS1	128	1	.	A	T	3.85	PASS	F	GT:GQ:DP:AF	1/1	20	49379	0.6853
OAS1	137	1	.	A	.	16.02	RefCall	F	GT:GQ:DP:AF	0/0	3	41	0.8537
OAS1	180	1	.	T	.	4.28	RefCall	F	GT:GQ:DP:AF	0/0	6	42	0.6667
OAS1	201	1	.	G	.	5.6	RefCall	F	GT:GQ:DP:AF	0/0	7	42	0.5476
OAS1	225	1	.	C	T	5.04	PASS	F	GT:GQ:DP:AF	1/1	21	49178	0.7256
OAS1	241	1	.	A	.	6.98	RefCall	F	GT:GQ:DP:AF	0/0	4	44	0.5455
OAS1	257	1	.	A	G	7.82	PASS	F	GT:GQ:DP:AF	0/1	23	49175	0.5181
OAS1	262	1	.	T	C	8.49	PASS	F	GT:GQ:DP:AF	0/1	23	49175	0.5002
OAS1	287	1	.	A	G	9.6	PASS	F	GT:GQ:DP:AF	0/1	28	49074	0.8637
OAS1	310	1	.	C	A	5.13	PASS	F	GT:GQ:DP:AF	0/1	18	48949	0.4033
OAS1	315	1	.	T	A	4.44	PASS	F	GT:GQ:DP:AF	0/1	27	48968	0.7608
OAS1	1562	1	.	C	.	7.69	RefCall	F	GT:GQ:DP:AF	0/0	16	45	0.4667
OAS1	2378	1	.	C	G	34.23	PASS	F	GT:GQ:DP:AF	1/1	33	48969	0.8794
OAS1	2821	1	.	A	C	36.94	PASS	F	GT:GQ:DP:AF	1/1	30	48969	0.8906
OAS1	3411	1	.	A	.	31.65	RefCall	F	GT:GQ:DP:AF	0/0	4	45	0.4
OAS1	4363	1	.	G	.	26.44	RefCall	F	GT:GQ:DP:AF	0/0	5	45	0.6889
OAS1	4400	1	.	G	.	25.1	RefCall	F	GT:GQ:DP:AF	0/0	5	44	0.7273
OAS1	4704	1	.	A	.	21.05	RefCall	F	GT:GQ:DP:AF	0/0	6	42	0.2381
OAS1	4750	1	.	G	.	35.21	RefCall	F	GT:GQ:DP:AF	0/0	7	42	0.5
OAS1	5005	1	.	A	.	30	RefCall	F	GT:GQ:DP:AF	0/0	8	42	0.5238
OAS1	5069	1	.	G	.	29.95	RefCall	F	GT:GQ:DP:AF	0/0	9	38	0.3421
OAS1	5188	1	.	G	.	29.46	RefCall	F	GT:GQ:DP:AF	0/0	5	30	0.5333
OAS1	5295	1	.	G	.	19.22	RefCall	F	GT:GQ:DP:AF	0/0	4	28	0.4286

OAS1	5331	1	.	T	.	21.65	RefCall	F	GT:GQ:DP:AF	0/0	7	45764	0.4183
OAS1	5381	1	.	T	.	29.11	RefCall	F	GT:GQ:DP:AF	0/0	34	45805	0.8069
OAS1	5971	1	.	C	.	32.02	RefCall	F	GT:GQ:DP:AF	0/0	36	45765	0.8851
OAS1	5992	1	.	G	A	29.63	PASS	F	GT:GQ:DP:AF	1/1	35	48854	0.9223
OAS1	6220	1	.	T	.	15.49	RefCall	F	GT:GQ:DP:AF	0/0	31	45825	0.7287
OAS1	6305	1	.	G	.	20.3	RefCall	F	GT:GQ:DP:AF	0/0	26	45912	0.8606
OAS1	6996	1	.	A	.	32.19	RefCall	F	GT:GQ:DP:AF	0/0	25	45917	0.8279
OAS1	7068	1	.	T	.	27.09	RefCall	F	GT:GQ:DP:AF	0/0	21	45999	0.638
OAS1	7426	1	.	T	.	7.54	RefCall	F	GT:GQ:DP:AF	0/0	35	46001	0.872
OAS1	7983	1	.	C	.	10.83	RefCall	F	GT:GQ:DP:AF	0/0	30	46024	0.8163
OAS1	8858	1	.	A	.	22.06	RefCall	F	GT:GQ:DP:AF	0/0	29	46480	0.8733
OAS1	9657	1	.	Gt	G	0	LowQual	F	GT:GQ:DP:AF	1/1	17	48689	0.4631
OAS1	9900	1	.	A	.	7.4	RefCall	F	GT:GQ:DP:AF	0/0	29	46513	0.9144
OAS1	10225	1	.	A	.	18.73	RefCall	F	GT:GQ:DP:AF	0/0	19	46407	0.6215
OAS1	10504	1	.	A	.	34.28	RefCall	F	GT:GQ:DP:AF	0/0	21	46004	0.6316
OAS1	10628	1	.	G	.	25.92	RefCall	F	GT:GQ:DP:AF	0/0	29	45994	0.7669
OAS1	12119	1	.	C	.	20.85	RefCall	F	GT:GQ:DP:AF	0/0	32	46064	0.8112
OAS1	12975	1	.	A	.	21.92	RefCall	F	GT:GQ:DP:AF	0/0	29	46054	0.9232
OAS1	13092	1	.	C	.	23.56	RefCall	F	GT:GQ:DP:AF	0/0	15	46111	0.6133
OAS1	13100	1	.	A	.	23.7	RefCall	F	GT:GQ:DP:AF	0/0	20	46128	0.6451
OAS1	13388	1	.	G	.	28.21	RefCall	F	GT:GQ:DP:AF	0/0	32	46346	0.9093
OAS1	13891	1	.	A	.	18.09	RefCall	F	GT:GQ:DP:AF	0/0	27	46361	0.9141
OAS1	13931	1	.	A	.	27.45	RefCall	F	GT:GQ:DP:AF	0/0	7	46425	0.3804
OAS1	13958	1	.	G	.	33.32	RefCall	F	GT:GQ:DP:AF	0/0	10	96899	0.7619
OAS1	13959	1	.	A	.	30.18	RefCall	F	GT:GQ:DP:AF	0/0	22	50350	0.5111
OAS1	14359	1	.	G	C	35.09	PASS	F	GT:GQ:DP:AF	1/1	24	48621	0.7266
OAS1	15055	1	.	T	.	17.47	RefCall	F	GT:GQ:DP:AF	0/0	0	50274	0.1163
OAS1	15178	1	.	G	.	24.93	RefCall	F	GT:GQ:DP:AF	0/0	7	50198	0.3263
OAS1	15228	1	.	A	G	33.36	PASS	F	GT:GQ:DP:AF	1/1	33	48402	0.9187
OAS1	33	2	.	C	.	5.39	RefCall	F	GT:GQ:DP:AF	0/0	7	12	0.9167
OAS1	34	2	.	T	.	6.1	RefCall	F	GT:GQ:DP:AF	0/0	8	12	0.9167
OAS1	43	2	.	T	C	7.39	PASS	F	GT:GQ:DP:AF	1/1	5	7	0.5714
OAS1	50	2	.	A	G	8.55	PASS	F	GT:GQ:DP:AF	1/1	6	7	0.1429
OAS1	57	2	.	T	C	5.89	PASS	F	GT:GQ:DP:AF	1/1	5	13	0.6923
OAS1	78	2	.	C	T	8.52	PASS	F	GT:GQ:DP:AF	0/1	8	13	0.3077
OAS1	79	2	.	A	G	4.72	PASS	F	GT:GQ:DP:AF	0/1	4	13	0.4615
OAS1	81	2	.	A	G	4.1	PASS	F	GT:GQ:DP:AF	0/1	8	13	0.5385
OAS1	88	2	.	G	A	8.2	PASS	F	GT:GQ:DP:AF	0/1	14	42630	0.3264
OAS1	106	2	.	G	A	5.56	PASS	F	GT:GQ:DP:AF	0/1	24	42476	0.7474
OAS1	136	2	.	C	T	7.24	PASS	F	GT:GQ:DP:AF	0/1	11	42465	0.7074
OAS1	165	2	.	A	G	8.08	PASS	F	GT:GQ:DP:AF	0/1	36	42376	0.9011

OAS1	169	2	.	A	G	8.29	PASS	F	GT:GQ:DP:AF	0/1	15	42021	0.6212
OAS1	178	2	.	T	C	6.31	PASS	F	GT:GQ:DP:AF	0/1	23	41918	0.5704
OAS1	180	2	.	T	C	5.1	PASS	F	GT:GQ:DP:AF	1/1	26	41899	0.7859
OAS1	181	2	.	A	G	5	PASS	F	GT:GQ:DP:AF	0/1	21	41025	0.5185
OAS1	200	2	.	T	C	9.19	PASS	F	GT:GQ:DP:AF	0/1	25	41029	0.4993
OAS1	201	2	.	G	A	8.27	PASS	F	GT:GQ:DP:AF	0/1	28	40760	0.7974
OAS1	257	2	.	A	G	6.76	PASS	F	GT:GQ:DP:AF	1/1	19	40683	0.4093
OAS1	259	2	.	G	.	16.22	RefCall	F	GT:GQ:DP:AF	0/0	4	13	0.5385
OAS1	286	2	.	C	T	8.6	PASS	F	GT:GQ:DP:AF	0/1	30	40692	0.7668
OAS1	294	2	.	T	C	8.55	PASS	F	GT:GQ:DP:AF	0/1	31	40689	0.8749
OAS1	295	2	.	G	A	9.77	PASS	F	GT:GQ:DP:AF	0/1	32	40690	0.8869
OAS1	310	2	.	C	A	6.48	PASS	F	GT:GQ:DP:AF	1/1	18	40653	0.6327
OAS1	315	2	.	T	A	5.53	PASS	F	GT:GQ:DP:AF	0/1	32	40566	0.7895
OAS1	319	2	.	T	A	4.82	PASS	F	GT:GQ:DP:AF	0/1	22	40313	0.4648
OAS1	1636	2	.	C	.	22.15	RefCall	F	GT:GQ:DP:AF	0/0	5	13	0.6154
OAS1	2121	2	.	C	.	29.84	RefCall	F	GT:GQ:DP:AF	0/0	7	15	0.3333
OAS1	2210	2	.	T	.	22.92	RefCall	F	GT:GQ:DP:AF	0/0	8	15	0.4
OAS1	2373	2	.	T	.	20.95	RefCall	F	GT:GQ:DP:AF	0/0	8	15	0.2667
OAS1	2748	2	.	C	.	22.82	RefCall	F	GT:GQ:DP:AF	0/0	6	15	0.3333
OAS1	2816	2	.	C	.	25.45	RefCall	F	GT:GQ:DP:AF	0/0	5	15	0.5333
OAS1	3189	2	.	G	.	26.97	RefCall	F	GT:GQ:DP:AF	0/0	5	15	0.5333
OAS1	3612	2	.	A	.	32.78	RefCall	F	GT:GQ:DP:AF	0/0	9	16	0.3125
OAS1	3769	2	.	C	.	26.68	RefCall	F	GT:GQ:DP:AF	0/0	8	16	0.25
OAS1	4337	2	.	A	.	28.97	RefCall	F	GT:GQ:DP:AF	0/0	6	16	0.6875
OAS1	4400	2	.	G	.	31.57	RefCall	F	GT:GQ:DP:AF	0/0	16	16	0.8125
OAS1	5069	2	.	G	.	28.94	RefCall	F	GT:GQ:DP:AF	0/0	8	16	0.5
OAS1	5188	2	.	G	.	30.2	RefCall	F	GT:GQ:DP:AF	0/0	8	16	0.5
OAS1	5295	2	.	G	.	24.5	RefCall	F	GT:GQ:DP:AF	0/0	9	16	0.3125
OAS1	5331	2	.	T	.	20.87	RefCall	F	GT:GQ:DP:AF	0/0	6	13	0.6923
OAS1	5381	2	.	T	.	28.02	RefCall	F	GT:GQ:DP:AF	0/0	5	12	0.5833
OAS1	5395	2	.	C	.	22.96	RefCall	F	GT:GQ:DP:AF	0/0	4	11	0.5455
OAS1	6215	2	.	A	.	26.2	RefCall	F	GT:GQ:DP:AF	0/0	22	49018	0.4676
OAS1	6996	2	.	A	.	30.98	RefCall	F	GT:GQ:DP:AF	0/0	29	49092	0.7867
OAS1	7068	2	.	T	.	30.14	RefCall	F	GT:GQ:DP:AF	0/0	22	49078	0.5537
OAS1	7226	2	.	T	.	29.35	RefCall	F	GT:GQ:DP:AF	0/0	20	49084	0.5162
OAS1	7695	2	.	C	.	22.31	RefCall	F	GT:GQ:DP:AF	0/0	22	49096	0.7379
OAS1	7983	2	.	C	.	26.1	RefCall	F	GT:GQ:DP:AF	0/0	25	49104	0.6997
OAS1	8858	2	.	A	.	20.61	RefCall	F	GT:GQ:DP:AF	0/0	26	49080	0.7566
OAS1	9900	2	.	A	.	14.24	RefCall	F	GT:GQ:DP:AF	0/0	32	48990	0.8026
OAS1	10215	2	.	A	.	24.29	RefCall	F	GT:GQ:DP:AF	0/0	26	48976	0.6493
OAS1	10225	2	.	A	.	11.97	RefCall	F	GT:GQ:DP:AF	0/0	28	48940	0.9159

OAS1	10504	2	.	A	.	36.87	RefCall	F	GT:GQ:DP:AF	0/0	31	48957	0.8208
OAS1	11183	2	.	C	.	15.07	RefCall	F	GT:GQ:DP:AF	0/0	28	49198	0.8758
OAS1	11405	2	.	A	.	23.51	RefCall	F	GT:GQ:DP:AF	0/0	30	49253	0.9124
OAS1	11419	2	.	G	.	26.31	RefCall	F	GT:GQ:DP:AF	0/0	24	49239	0.6192
OAS1	13092	2	.	C	.	21.9	RefCall	F	GT:GQ:DP:AF	0/0	20	49062	0.6273
OAS1	13100	2	.	A	.	25.68	RefCall	F	GT:GQ:DP:AF	0/0	28	49079	0.7646
OAS1	13688	2	.	C	.	28.16	RefCall	F	GT:GQ:DP:AF	0/0	22	49081	0.4673
OAS1	13891	2	.	A	.	19.52	RefCall	F	GT:GQ:DP:AF	0/0	26	49140	0.7756
OAS1	13931	2	.	A	.	30.16	RefCall	F	GT:GQ:DP:AF	0/0	30	49293	0.9081
OAS1	13958	2	.	G	.	31.91	RefCall	F	GT:GQ:DP:AF	0/0	30	49309	0.9134
OAS1	13959	2	.	A	.	32.64	RefCall	F	GT:GQ:DP:AF	0/0	29	49330	0.9127
OAS1	14108	2	.	C	.	18.65	RefCall	F	GT:GQ:DP:AF	0/0	22	92021	0.6894
OAS1	14345	2	.	C	.	32.28	RefCall	F	GT:GQ:DP:AF	0/0	26	92154	0.7575
OAS1	15055	2	.	T	.	22.48	RefCall	F	GT:GQ:DP:AF	0/0	20	42851	0.5115
OAS1	57	3	.	T	C	6.01	PASS	F	GT:GQ:DP:AF	1/1	6	34	0.7647
OAS1	66	3	.	G	.	7.76	RefCall	F	GT:GQ:DP:AF	0/0	7	34	0.7059
OAS1	81	3	.	A	G	7.81	PASS	F	GT:GQ:DP:AF	1/1	7	34	0.7941
OAS1	87	3	.	C	T	5.68	PASS	F	GT:GQ:DP:AF	0/1	9	41	0.4634
OAS1	114	3	.	A	C	10.68	PASS	F	GT:GQ:DP:AF	1/1	21	30398	0.5136
OAS1	136	3	.	C	T	6.64	PASS	F	GT:GQ:DP:AF	0/1	9	30347	0.4274
OAS1	137	3	.	A	.	6.51	RefCall	F	GT:GQ:DP:AF	0/0	5	37	0.4595
OAS1	145	3	.	A	T	9.02	PASS	F	GT:GQ:DP:AF	0/1	0	30311	0.0983
OAS1	201	3	.	G	.	7.23	RefCall	F	GT:GQ:DP:AF	0/0	10	39	0.7692
OAS1	213	3	.	T	.	6.97	RefCall	F	GT:GQ:DP:AF	0/0	6	40	0.575
OAS1	219	3	.	G	A	5.71	PASS	F	GT:GQ:DP:AF	0/1	4	30310	0.444
OAS1	225	3	.	C	T	6.75	PASS	F	GT:GQ:DP:AF	1/1	9	30280	0.3319
OAS1	238	3	.	G	.	4.3	RefCall	F	GT:GQ:DP:AF	0/0	6	40	0.325
OAS1	241	3	.	A	G	1.53	LowQual	F	GT:GQ:DP:AF	1/1	17	30161	0.7099
OAS1	257	3	.	A	G	6.91	PASS	F	GT:GQ:DP:AF	0/1	19	29804	0.5675
OAS1	258	3	.	A	T	6.31	PASS	F	GT:GQ:DP:AF	0/1	21	29797	0.7889
OAS1	264	3	.	T	C	6.12	PASS	F	GT:GQ:DP:AF	0/1	21	29519	0.6828
OAS1	275	3	.	C	T	6.6	PASS	F	GT:GQ:DP:AF	0/1	17	29336	0.5181
OAS1	286	3	.	C	T	5.96	PASS	F	GT:GQ:DP:AF	0/1	15	29342	0.503
OAS1	287	3	.	A	G	9.7	PASS	F	GT:GQ:DP:AF	0/1	22	29257	0.8986
OAS1	295	3	.	G	A	7.05	PASS	F	GT:GQ:DP:AF	0/1	23	29186	0.794
OAS1	310	3	.	C	A	10.26	PASS	F	GT:GQ:DP:AF	1/1	21	29155	0.6671
OAS1	1636	3	.	C	.	18.3	RefCall	F	GT:GQ:DP:AF	0/0	7	41	0.5366
OAS1	2821	3	.	A	C	36.11	PASS	F	GT:GQ:DP:AF	1/1	22	29144	0.7677
OAS1	3189	3	.	G	.	25.69	RefCall	F	GT:GQ:DP:AF	0/0	6	41	0.4634
OAS1	3612	3	.	A	.	28.61	RefCall	F	GT:GQ:DP:AF	0/0	5	41	0.5854
OAS1	4344	3	.	T	.	24.8	RefCall	F	GT:GQ:DP:AF	0/0	6	41	0.8293

OAS1	4363	3	.	G	.	21.47	RefCall	F	GT:GQ:DP:AF	0/0	4	41	0.4878
OAS1	4400	3	.	G	.	27.49	RefCall	F	GT:GQ:DP:AF	0/0	1	41	0.3415
OAS1	4704	3	.	A	.	23.96	RefCall	F	GT:GQ:DP:AF	0/0	6	41	0.7561
OAS1	5005	3	.	A	.	25.72	RefCall	F	GT:GQ:DP:AF	0/0	6	41	0.1951
OAS1	5188	3	.	G	.	25.91	RefCall	F	GT:GQ:DP:AF	0/0	6	41	0.6585
OAS1	5295	3	.	G	.	23.89	RefCall	F	GT:GQ:DP:AF	0/0	6	41	0.7073
OAS1	5303	3	.	G	.	24.75	RefCall	F	GT:GQ:DP:AF	0/0	5	37	0.3514
OAS1	5331	3	.	T	.	23.69	RefCall	F	GT:GQ:DP:AF	0/0	9	29	0.4483
OAS1	5381	3	.	T	.	24.27	RefCall	F	GT:GQ:DP:AF	0/0	7	28	0.25
OAS1	5774	3	.	G	.	31.65	RefCall	F	GT:GQ:DP:AF	0/0	10	20	0.55
OAS1	5776	3	.	C	.	27.91	RefCall	F	GT:GQ:DP:AF	0/0	18	43128	0.4711
OAS1	5971	3	.	C	.	29.73	RefCall	F	GT:GQ:DP:AF	0/0	36	43159	0.8879
OAS1	6220	3	.	T	.	15.94	RefCall	F	GT:GQ:DP:AF	0/0	25	43146	0.7542
OAS1	6221	3	.	G	.	6.67	RefCall	F	GT:GQ:DP:AF	0/0	28	43082	0.8005
OAS1	6305	3	.	G	.	22.58	RefCall	F	GT:GQ:DP:AF	0/0	24	43102	0.8836
OAS1	6996	3	.	A	.	30.69	RefCall	F	GT:GQ:DP:AF	0/0	21	43111	0.8607
OAS1	7068	3	.	T	.	25.13	RefCall	F	GT:GQ:DP:AF	0/0	27	43111	0.8249
OAS1	7226	3	.	T	.	26.92	RefCall	F	GT:GQ:DP:AF	0/0	23	43124	0.6354
OAS1	7426	3	.	T	.	20.35	RefCall	F	GT:GQ:DP:AF	0/0	25	43119	0.8182
OAS1	7983	3	.	C	.	17.6	RefCall	F	GT:GQ:DP:AF	0/0	25	43488	0.9138
OAS1	8642	3	.	T	G	5.23	PASS	F	GT:GQ:DP:AF	0/1	26	29134	0.881
OAS1	8858	3	.	A	.	21.6	RefCall	F	GT:GQ:DP:AF	0/0	23	43433	0.6199
OAS1	9281	3	.	T	C	9.2	PASS	F	GT:GQ:DP:AF	0/1	26	29134	0.8907
OAS1	9657	3	.	Gt	G	0.7	LowQual	F	GT:GQ:DP:AF	1/1	21	29089	0.7505
OAS1	9830	3	.	C	A	4.97	PASS	F	GT:GQ:DP:AF	0/1	8	29049	0.4502
OAS1	9900	3	.	A	.	9.37	RefCall	F	GT:GQ:DP:AF	0/0	24	43403	0.5636
OAS1	10225	3	.	A	.	17.41	RefCall	F	GT:GQ:DP:AF	0/0	23	43134	0.6287
OAS1	11405	3	.	A	.	19.18	RefCall	F	GT:GQ:DP:AF	0/0	24	43133	0.7618
OAS1	11419	3	.	G	.	21.93	RefCall	F	GT:GQ:DP:AF	0/0	31	43156	0.8906
OAS1	12119	3	.	C	.	21.96	RefCall	F	GT:GQ:DP:AF	0/0	27	43155	0.6945
OAS1	13092	3	.	C	.	17.45	RefCall	F	GT:GQ:DP:AF	0/0	29	43152	0.8256
OAS1	13100	3	.	A	.	15.96	RefCall	F	GT:GQ:DP:AF	0/0	15	43168	0.6237
OAS1	13365	3	.	A	.	22.45	RefCall	F	GT:GQ:DP:AF	0/0	6	43168	0.67
OAS1	13688	3	.	C	.	23.34	RefCall	F	GT:GQ:DP:AF	0/0	22	43204	0.6463
OAS1	13857	3	.	T	.	21.76	RefCall	F	GT:GQ:DP:AF	0/0	30	43348	0.9086
OAS1	13931	3	.	A	.	22.47	RefCall	F	GT:GQ:DP:AF	0/0	25	43361	0.9114
OAS1	13958	3	.	G	.	26.61	RefCall	F	GT:GQ:DP:AF	0/0	26	43404	0.9108
OAS1	13959	3	.	A	.	26.01	RefCall	F	GT:GQ:DP:AF	0/0	20	43443	0.3838
OAS1	14211	3	.	A	.	21.93	RefCall	F	GT:GQ:DP:AF	0/0	17	73805	0.7606
OAS1	14331	3	.	G	A	8.92	PASS	F	GT:GQ:DP:AF	0/1	25	29042	0.7964
OAS1	14345	3	.	C	.	25.56	RefCall	F	GT:GQ:DP:AF	0/0	5	73848	0.4467

OAS1	15228	3	.	A	G	4.91	PASS	F	GT:GQ:DP:AF	0/1	4	28652	0.4578
OAS1	50	4	.	A	G	5.81	PASS	F	GT:GQ:DP:AF	0/1	5	48	0.7917
OAS1	57	4	.	T	C	5.76	PASS	F	GT:GQ:DP:AF	1/1	5	54	0.7963
OAS1	81	4	.	A	G	11.42	PASS	F	GT:GQ:DP:AF	1/1	11	55	0.7455
OAS1	82	4	.	T	.	8.81	RefCall	F	GT:GQ:DP:AF	0/0	11	55	0.4545
OAS1	87	4	.	C	T	8.38	PASS	F	GT:GQ:DP:AF	0/1	8	55	0.6182
OAS1	96	4	.	C	T	11.21	PASS	F	GT:GQ:DP:AF	0/1	8	55	0.4545
OAS1	127	4	.	C	G	8.49	PASS	F	GT:GQ:DP:AF	1/1	9	55	0.3818
OAS1	145	4	.	A	T	9.65	PASS	F	GT:GQ:DP:AF	0/1	11	44308	0.3812
OAS1	180	4	.	T	C	11.54	PASS	F	GT:GQ:DP:AF	1/1	26	105598	0.7598
OAS1	201	4	.	G	C	8.47	PASS	F	GT:GQ:DP:AF	0/1	40	105674	0.8983
OAS1	207	4	.	C	.	7.52	RefCall	F	GT:GQ:DP:AF	0/0	8	55	0.5273
OAS1	223	4	.	A	G	8.15	PASS	F	GT:GQ:DP:AF	0/1	15	61079	0.3937
OAS1	225	4	.	C	T	9.93	PASS	F	GT:GQ:DP:AF	1/1	0	60914	0.2146
OAS1	241	4	.	A	G	1.95	LowQual	F	GT:GQ:DP:AF	0/1	23	60839	0.4496
OAS1	256	4	.	T	C	4.7	PASS	F	GT:GQ:DP:AF	0/1	3	60791	0.3261
OAS1	258	4	.	A	T	14.8	PASS	F	GT:GQ:DP:AF	0/1	11	60643	0.7106
OAS1	264	4	.	T	C	6.1	PASS	F	GT:GQ:DP:AF	0/1	13	60235	0.6186
OAS1	275	4	.	C	T	5.45	PASS	F	GT:GQ:DP:AF	1/1	26	60091	0.7888
OAS1	287	4	.	A	G	11.05	PASS	F	GT:GQ:DP:AF	0/1	28	59805	0.6826
OAS1	295	4	.	G	A	6.43	PASS	F	GT:GQ:DP:AF	0/1	20	59496	0.7226
OAS1	300	4	.	C	T	8.89	PASS	F	GT:GQ:DP:AF	0/1	24	59483	0.5121
OAS1	310	4	.	C	A	7.83	PASS	F	GT:GQ:DP:AF	1/1	28	59464	0.4703
OAS1	319	4	.	T	A	3.55	PASS	F	GT:GQ:DP:AF	1/1	32	59329	0.861
OAS1	1636	4	.	C	.	18.43	RefCall	F	GT:GQ:DP:AF	0/0	11	57	0.5965
OAS1	2210	4	.	T	.	12.7	RefCall	F	GT:GQ:DP:AF	0/0	8	58	0.2069
OAS1	2821	4	.	A	C	37.2	PASS	F	GT:GQ:DP:AF	1/1	31	59244	0.7943
OAS1	3612	4	.	A	.	21.87	RefCall	F	GT:GQ:DP:AF	0/0	7	58	0.7759
OAS1	3769	4	.	C	.	21.64	RefCall	F	GT:GQ:DP:AF	0/0	8	56	0.4464
OAS1	4337	4	.	A	.	28.84	RefCall	F	GT:GQ:DP:AF	0/0	9	56	0.8036
OAS1	4363	4	.	G	.	27.07	RefCall	F	GT:GQ:DP:AF	0/0	1	56	0.3929
OAS1	4400	4	.	G	.	27.65	RefCall	F	GT:GQ:DP:AF	0/0	4	56	0.4464
OAS1	4678	4	.	A	.	32.21	RefCall	F	GT:GQ:DP:AF	0/0	14	56	0.2857
OAS1	4704	4	.	A	.	24.78	RefCall	F	GT:GQ:DP:AF	0/0	6	54	0.5556
OAS1	4741	4	.	C	.	33.97	RefCall	F	GT:GQ:DP:AF	0/0	5	53	0.7358
OAS1	4750	4	.	G	.	34.96	RefCall	F	GT:GQ:DP:AF	0/0	11	44	0.3864
OAS1	4891	4	.	Ga	G	6.52	PASS	F	GT:GQ:DP:AF	0/1	28	59184	0.7602
OAS1	5005	4	.	A	.	28.78	RefCall	F	GT:GQ:DP:AF	0/0	6	43	0.2791
OAS1	5069	4	.	G	.	27.22	RefCall	F	GT:GQ:DP:AF	0/0	8	43	0.3256
OAS1	5188	4	.	G	.	31.71	RefCall	F	GT:GQ:DP:AF	0/0	7	35	0.6
OAS1	5271	4	.	G	A	27.35	PASS	F	GT:GQ:DP:AF	0/1	33	59211	0.878

OAS1	5295	4	.	G	.	18.77	RefCall	F	GT:GQ:DP:AF	0/0	3	24	0.625
OAS1	5331	4	.	T	.	18.19	RefCall	F	GT:GQ:DP:AF	0/0	18	43564	0.4669
OAS1	5381	4	.	T	.	29.83	RefCall	F	GT:GQ:DP:AF	0/0	12	43618	0.5553
OAS1	5395	4	.	C	.	18.28	RefCall	F	GT:GQ:DP:AF	0/0	37	43631	0.8871
OAS1	5415	4	.	A	.	24.22	RefCall	F	GT:GQ:DP:AF	0/0	21	43629	0.8011
OAS1	5776	4	.	C	.	30.18	RefCall	F	GT:GQ:DP:AF	0/0	21	43639	0.6437
OAS1	6215	4	.	A	.	26.11	RefCall	F	GT:GQ:DP:AF	0/0	28	43638	0.9179
OAS1	6220	4	.	T	.	16.15	RefCall	F	GT:GQ:DP:AF	0/0	27	43641	0.8624
OAS1	6305	4	.	G	.	25.33	RefCall	F	GT:GQ:DP:AF	0/0	27	43654	0.8285
OAS1	7068	4	.	T	.	27.13	RefCall	F	GT:GQ:DP:AF	0/0	32	43679	0.8471
OAS1	7101	4	.	C	.	32.75	RefCall	F	GT:GQ:DP:AF	0/0	24	43676	0.6414
OAS1	7426	4	.	T	.	11.37	RefCall	F	GT:GQ:DP:AF	0/0	33	43665	0.8877
OAS1	7983	4	.	C	.	26.97	RefCall	F	GT:GQ:DP:AF	0/0	34	43664	0.8733
OAS1	8642	4	.	T	G	40.21	PASS	F	GT:GQ:DP:AF	1/1	25	59129	0.7489
OAS1	8858	4	.	A	.	15.4	RefCall	F	GT:GQ:DP:AF	0/0	6	43695	0.2212
OAS1	9657	4	.	Gtt	G,Gt	0	LowQual	F	GT:GQ:DP:AF	1/2	35	59089	0.9225
OAS1	9830	4	.	C	A	23.34	PASS	F	GT:GQ:DP:AF	0/1	21	58765	0.6004
OAS1	9900	4	.	A	.	3.73	RefCall	F	GT:GQ:DP:AF	0/0	28	43719	0.8174
OAS1	10225	4	.	A	.	11.05	RefCall	F	GT:GQ:DP:AF	0/0	27	44175	0.8744
OAS1	11183	4	.	C	.	13.12	RefCall	F	GT:GQ:DP:AF	0/0	31	44224	0.9157
OAS1	11419	4	.	G	.	26.41	RefCall	F	GT:GQ:DP:AF	0/0	27	44237	0.4899
OAS1	12119	4	.	C	.	28.53	RefCall	F	GT:GQ:DP:AF	0/0	18	44190	0.6251
OAS1	12975	4	.	A	.	20.86	RefCall	F	GT:GQ:DP:AF	0/0	18	43810	0.6291
OAS1	13092	4	.	C	.	24.1	RefCall	F	GT:GQ:DP:AF	0/0	29	43835	0.7642
OAS1	13157	4	.	A	G	28.36	PASS	F	GT:GQ:DP:AF	0/1	11	58766	0.4642
OAS1	13388	4	.	G	.	32.42	RefCall	F	GT:GQ:DP:AF	0/0	18	43833	0.4683
OAS1	13688	4	.	C	.	31.88	RefCall	F	GT:GQ:DP:AF	0/0	24	43837	0.7302
OAS1	13931	4	.	A	.	28.09	RefCall	F	GT:GQ:DP:AF	0/0	30	43859	0.6929
OAS1	13958	4	.	G	.	33.76	RefCall	F	GT:GQ:DP:AF	0/0	26	43934	0.7723
OAS1	14211	4	.	A	.	25.18	RefCall	F	GT:GQ:DP:AF	0/0	16	43934	0.6134
OAS1	14359	4	.	G	C	35.75	PASS	F	GT:GQ:DP:AF	1/1	28	58643	0.7464
OAS1	15045	4	.	A	.	21.82	RefCall	F	GT:GQ:DP:AF	0/0	25	43991	0.6424
OAS1	15055	4	.	T	.	11.23	RefCall	F	GT:GQ:DP:AF	0/0	27	44221	0.9113
OAS1	15178	4	.	G	.	28.67	RefCall	F	GT:GQ:DP:AF	0/0	32	44255	0.8069
OAS1	15228	4	.	A	G	31.94	PASS	F	GT:GQ:DP:AF	1/1	31	58300	0.9173
OAS1	34	5	.	T	.	8.69	RefCall	F	GT:GQ:DP:AF	0/0	4	33	0.5152
OAS1	50	5	.	A	G	3.22	PASS	F	GT:GQ:DP:AF	0/1	8	12	0.0833
OAS1	57	5	.	T	C	6.76	PASS	F	GT:GQ:DP:AF	1/1	3	28	0.7857
OAS1	73	5	.	T	C	2.7	PASS	F	GT:GQ:DP:AF	1/1	6	30	0.8
OAS1	74	5	.	G	.	4.81	RefCall	F	GT:GQ:DP:AF	0/0	5	33	0.4242
OAS1	79	5	.	A	G	6.59	PASS	F	GT:GQ:DP:AF	0/1	2	32	0.4688

OAS1	82	5	.	T	.	4.24	RefCall	F	GT:GQ:DP:AF	0/0	8	35	0.6571
OAS1	88	5	.	G	A	5.44	PASS	F	GT:GQ:DP:AF	0/1	4	32	0.5938
OAS1	125	5	.	T	.	8.76	RefCall	F	GT:GQ:DP:AF	0/0	8	35	0.4571
OAS1	127	5	.	C	G	8.27	PASS	F	GT:GQ:DP:AF	1/1	6	33	0.2727
OAS1	169	5	.	A	.	7.03	RefCall	F	GT:GQ:DP:AF	0/0	7	36	0.5556
OAS1	201	5	.	G	A	6.41	PASS	F	GT:GQ:DP:AF	0/1	28	51849	0.7624
OAS1	264	5	.	T	C	4.45	PASS	F	GT:GQ:DP:AF	0/1	34	51853	0.8792
OAS1	287	5	.	A	G	10.39	PASS	F	GT:GQ:DP:AF	0/1	31	51854	0.8901
OAS1	319	5	.	T	.	5.03	RefCall	F	GT:GQ:DP:AF	0/0	6	36	0.3333
OAS1	325	5	.	T	.	19.27	RefCall	F	GT:GQ:DP:AF	0/0	4	34	0.4118
OAS1	327	5	.	T	.	13.47	RefCall	F	GT:GQ:DP:AF	0/0	10	31	0.3871
OAS1	345	5	.	T	.	12.72	RefCall	F	GT:GQ:DP:AF	0/0	5	20	0.5
OAS1	1636	5	.	C	.	15.8	RefCall	F	GT:GQ:DP:AF	0/0	19	5	0.8
OAS1	2373	5	.	T	.	19.65	RefCall	F	GT:GQ:DP:AF	0/0	13	5	0.8
OAS1	2733	5	.	C	.	25.07	RefCall	F	GT:GQ:DP:AF	0/0	12	2	0
OAS1	2748	5	.	C	.	27.59	RefCall	F	GT:GQ:DP:AF	0/0	15	42925	0.4649
OAS1	2821	5	.	A	C	16.04	PASS	F	GT:GQ:DP:AF	0/1	1	51818	0.2942
OAS1	3189	5	.	G	.	21.8	RefCall	F	GT:GQ:DP:AF	0/0	19	42934	0.5426
OAS1	3612	5	.	A	.	26.65	RefCall	F	GT:GQ:DP:AF	0/0	25	42914	0.7752
OAS1	3769	5	.	C	.	28.26	RefCall	F	GT:GQ:DP:AF	0/0	27	42914	0.7381
OAS1	4337	5	.	A	.	28.28	RefCall	F	GT:GQ:DP:AF	0/0	16	42939	0.4679
OAS1	4363	5	.	G	.	24.29	RefCall	F	GT:GQ:DP:AF	0/0	21	42900	0.7546
OAS1	4400	5	.	G	.	26.77	RefCall	F	GT:GQ:DP:AF	0/0	26	42835	0.8024
OAS1	4704	5	.	A	.	27.2	RefCall	F	GT:GQ:DP:AF	0/0	28	42846	0.6433
OAS1	5069	5	.	G	.	25.62	RefCall	F	GT:GQ:DP:AF	0/0	28	42876	0.9197
OAS1	5188	5	.	G	.	28.12	RefCall	F	GT:GQ:DP:AF	0/0	24	42885	0.8579
OAS1	5295	5	.	G	.	20.03	RefCall	F	GT:GQ:DP:AF	0/0	26	42876	0.8283
OAS1	5303	5	.	G	.	17.64	RefCall	F	GT:GQ:DP:AF	0/0	27	42937	0.6416
OAS1	5331	5	.	T	.	22.28	RefCall	F	GT:GQ:DP:AF	0/0	25	43436	0.8704
OAS1	5381	5	.	T	.	28.25	RefCall	F	GT:GQ:DP:AF	0/0	28	43448	0.9133
OAS1	5395	5	.	C	.	25.14	RefCall	F	GT:GQ:DP:AF	0/0	20	43384	0.6191
OAS1	5415	5	.	A	.	28.89	RefCall	F	GT:GQ:DP:AF	0/0	17	43361	0.5628
OAS1	5776	5	.	C	.	27.13	RefCall	F	GT:GQ:DP:AF	0/0	22	43045	0.6283
OAS1	6042	5	.	C	.	32.55	RefCall	F	GT:GQ:DP:AF	0/0	28	43049	0.7628
OAS1	6220	5	.	T	.	20.77	RefCall	F	GT:GQ:DP:AF	0/0	25	43054	0.4638
OAS1	6305	5	.	G	.	25	RefCall	F	GT:GQ:DP:AF	0/0	28	43066	0.7338
OAS1	6996	5	.	A	.	30.89	RefCall	F	GT:GQ:DP:AF	0/0	27	43082	0.6907
OAS1	7101	5	.	C	.	33.39	RefCall	F	GT:GQ:DP:AF	0/0	32	43108	0.8449
OAS1	7227	5	.	A	.	6.96	RefCall	F	GT:GQ:DP:AF	0/0	20	43133	0.6136
OAS1	7426	5	.	T	.	8.17	RefCall	F	GT:GQ:DP:AF	0/0	25	43175	0.6484
OAS1	7695	5	.	C	.	21.42	RefCall	F	GT:GQ:DP:AF	0/0	30	43375	0.9084

OAS1	8131	5	.	T	.	30.77	RefCall	F	GT:GQ:DP:AF	0/0	33	43427	0.8078
OAS1	8580	5	.	A	.	27.89	RefCall	F	GT:GQ:DP:AF	0/0	6	43464	0.5072
OAS1	8642	5	.	T	G	39.69	PASS	F	GT:GQ:DP:AF	1/1	34	51766	0.9219
OAS1	9246	5	.	A	.	9.73	RefCall	F	GT:GQ:DP:AF	0/0	8	43528	0.3821
OAS1	9657	5	.	Gtt	G	0	LowQual	F	GT:GQ:DP:AF	0/1	25	51735	0.7283
OAS1	9900	5	.	A	.	1.93	RefCall	F	GT:GQ:DP:AF	0/0	21	96759	0.6847
OAS1	10225	5	.	A	.	20.73	RefCall	F	GT:GQ:DP:AF	0/0	30	97024	0.8032
OAS1	10504	5	.	A	.	27.77	RefCall	F	GT:GQ:DP:AF	0/0	27	97101	0.6955
OAS1	11183	5	.	C	.	16.03	RefCall	F	GT:GQ:DP:AF	0/0	39	97087	0.8971
OAS1	11756	5	.	T	.	20.21	RefCall	F	GT:GQ:DP:AF	0/0	9	53346	0.4558
OAS1	12119	5	.	C	.	25.87	RefCall	F	GT:GQ:DP:AF	0/0	0	53294	0.1262
OAS1	12894	5	.	G	.	29.96	RefCall	F	GT:GQ:DP:AF	0/0	1	53233	0.3253
OAS1	13092	5	.	C	.	17.29	RefCall	F	GT:GQ:DP:AF	0/0	20	53042	0.7105
OAS1	13100	5	.	A	.	21.2	RefCall	F	GT:GQ:DP:AF	0/0	27	52952	0.9006
OAS1	13688	5	.	C	.	30.87	RefCall	F	GT:GQ:DP:AF	0/0	16	52630	0.6246
OAS1	13931	5	.	A	.	28.26	RefCall	F	GT:GQ:DP:AF	0/0	20	52424	0.6839
OAS1	13958	5	.	G	.	34.5	RefCall	F	GT:GQ:DP:AF	0/0	25	52239	0.6863
OAS1	13959	5	.	A	.	31.9	RefCall	F	GT:GQ:DP:AF	0/0	29	52040	0.7878
OAS1	14108	5	.	Ca	C	1.98	LowQual	F	GT:GQ:DP:AF	0/1	16	51566	0.4608
OAS1	14359	5	.	G	C	34.3	PASS	F	GT:GQ:DP:AF	1/1	24	51498	0.7505
OAS1	14495	5	.	T	.	25.2	RefCall	F	GT:GQ:DP:AF	0/0	17	52038	0.5193
OAS1	15055	5	.	T	.	16.27	RefCall	F	GT:GQ:DP:AF	0/0	21	52041	0.4988
OAS1	15178	5	.	G	.	24.79	RefCall	F	GT:GQ:DP:AF	0/0	30	51923	0.794
OAS1	15228	5	.	A	G	32.26	PASS	F	GT:GQ:DP:AF	1/1	32	51235	0.9159
OAS1	33	6	.	C	.	8.11	RefCall	F	GT:GQ:DP:AF	0/0	9	44	0.3864
OAS1	34	6	.	T	.	7.92	RefCall	F	GT:GQ:DP:AF	0/0	10	45	0.4667
OAS1	50	6	.	A	G	10.22	PASS	F	GT:GQ:DP:AF	1/1	8	16	0.6875
OAS1	57	6	.	T	C	2.96	PASS	F	GT:GQ:DP:AF	1/1	7	16	0
OAS1	74	6	.	G	.	7.3	RefCall	F	GT:GQ:DP:AF	0/0	0	45	0.3556
OAS1	81	6	.	A	G	4.8	PASS	F	GT:GQ:DP:AF	0/1	10	40	0.85
OAS1	96	6	.	C	T	9.45	PASS	F	GT:GQ:DP:AF	0/1	2	44	0.75
OAS1	105	6	.	C	T	4.13	PASS	F	GT:GQ:DP:AF	0/1	7	44	0.7273
OAS1	106	6	.	G	A	9.03	PASS	F	GT:GQ:DP:AF	0/1	4	44	0.6818
OAS1	127	6	.	C	G	10.88	PASS	F	GT:GQ:DP:AF	1/1	9	44	0.3864
OAS1	128	6	.	A	T	0.85	LowQual	F	GT:GQ:DP:AF	1/1	4	44	0.4318
OAS1	153	6	.	C	.	6.28	RefCall	F	GT:GQ:DP:AF	0/0	4	47	0.5106
OAS1	165	6	.	A	.	4.93	RefCall	F	GT:GQ:DP:AF	0/0	6	48	0.5
OAS1	180	6	.	T	C	6.58	PASS	F	GT:GQ:DP:AF	1/1	6	47	0.5957
OAS1	200	6	.	T	C	6.42	PASS	F	GT:GQ:DP:AF	0/1	4	47	0.2979
OAS1	214	6	.	G	.	4.87	RefCall	F	GT:GQ:DP:AF	0/0	6	49	0.3265
OAS1	241	6	.	A	.	4.99	RefCall	F	GT:GQ:DP:AF	0/0	4	49	0.6122

OAS1	256	6	.	T	C	4.44	PASS	F	GT:GQ:DP:AF	0/1	35	53413	0.9007
OAS1	258	6	.	A	T	11.82	PASS	F	GT:GQ:DP:AF	0/1	17	53105	0.6194
OAS1	262	6	.	T	C	5.69	PASS	F	GT:GQ:DP:AF	1/1	31	52860	0.6835
OAS1	263	6	.	G	A	9.11	PASS	F	GT:GQ:DP:AF	0/1	23	52647	0.7241
OAS1	265	6	.	G	A	11.24	PASS	F	GT:GQ:DP:AF	0/1	18	52609	0.5187
OAS1	287	6	.	A	G	5.58	PASS	F	GT:GQ:DP:AF	0/1	24	52611	0.5024
OAS1	294	6	.	T	.	5.2	RefCall	F	GT:GQ:DP:AF	0/0	4	45	0.3778
OAS1	295	6	.	G	.	6.26	RefCall	F	GT:GQ:DP:AF	0/0	11	44	0.3636
OAS1	300	6	.	C	T	8.06	PASS	F	GT:GQ:DP:AF	0/1	19	52337	0.405
OAS1	310	6	.	C	A	6.53	PASS	F	GT:GQ:DP:AF	1/1	30	52344	0.7613
OAS1	1636	6	.	C	.	15.99	RefCall	F	GT:GQ:DP:AF	0/0	5	44	0.6136
OAS1	2210	6	.	T	.	19.45	RefCall	F	GT:GQ:DP:AF	0/0	9	44	0.3864
OAS1	2378	6	.	C	G	30.81	PASS	F	GT:GQ:DP:AF	1/1	34	52346	0.8807
OAS1	2387	6	.	C	.	34.65	RefCall	F	GT:GQ:DP:AF	0/0	11	43	0.4186
OAS1	2821	6	.	A	C	38.32	PASS	F	GT:GQ:DP:AF	1/1	18	52330	0.6356
OAS1	3189	6	.	G	.	28.6	RefCall	F	GT:GQ:DP:AF	0/0	5	37	0.3243
OAS1	3612	6	.	A	.	25.96	RefCall	F	GT:GQ:DP:AF	0/0	5	35	0.6857
OAS1	3769	6	.	C	.	24.92	RefCall	F	GT:GQ:DP:AF	0/0	6	35	0.7429
OAS1	4363	6	.	G	.	23.5	RefCall	F	GT:GQ:DP:AF	0/0	8	32	0.3438
OAS1	4400	6	.	G	.	27.33	RefCall	F	GT:GQ:DP:AF	0/0	6	24	0.7083
OAS1	4704	6	.	A	.	23.32	RefCall	F	GT:GQ:DP:AF	0/0	15	42138	0.4698
OAS1	4750	6	.	G	.	33.86	RefCall	F	GT:GQ:DP:AF	0/0	19	42201	0.5549
OAS1	4886	6	.	G	.	29.99	RefCall	F	GT:GQ:DP:AF	0/0	30	42204	0.8092
OAS1	5005	6	.	A	.	29.23	RefCall	F	GT:GQ:DP:AF	0/0	34	42207	0.8063
OAS1	5069	6	.	G	.	26.59	RefCall	F	GT:GQ:DP:AF	0/0	38	42191	0.886
OAS1	5188	6	.	G	.	27.79	RefCall	F	GT:GQ:DP:AF	0/0	28	42158	0.7563
OAS1	5295	6	.	G	.	23.14	RefCall	F	GT:GQ:DP:AF	0/0	25	42164	0.8037
OAS1	5303	6	.	G	.	24.52	RefCall	F	GT:GQ:DP:AF	0/0	24	42146	0.6521
OAS1	5331	6	.	T	.	20.82	RefCall	F	GT:GQ:DP:AF	0/0	23	42146	0.8627
OAS1	6305	6	.	G	.	26.18	RefCall	F	GT:GQ:DP:AF	0/0	27	42156	0.8284
OAS1	6996	6	.	A	.	29.92	RefCall	F	GT:GQ:DP:AF	0/0	23	42213	0.6397
OAS1	7068	6	.	T	.	24.51	RefCall	F	GT:GQ:DP:AF	0/0	33	42204	0.8738
OAS1	7227	6	.	A	G	33.37	PASS	F	GT:GQ:DP:AF	1/1	27	52279	0.8046
OAS1	7983	6	.	C	.	22.49	RefCall	F	GT:GQ:DP:AF	0/0	29	42208	0.8734
OAS1	8580	6	.	A	.	26.41	RefCall	F	GT:GQ:DP:AF	0/0	29	42228	0.8174
OAS1	8858	6	.	A	.	1.55	RefCall	F	GT:GQ:DP:AF	0/0	26	42729	0.8711
OAS1	9246	6	.	A	.	2.1	RefCall	F	GT:GQ:DP:AF	0/0	27	42751	0.9139
OAS1	9657	6	.	Gtt	G,Gt	0	LowQual	F	GT:GQ:DP:AF	1/2	34	52263	0.9232
OAS1	9826	6	.	A	.	26.72	RefCall	F	GT:GQ:DP:AF	0/0	23	42691	0.6169
OAS1	9900	6	.	A	.	11.67	RefCall	F	GT:GQ:DP:AF	0/0	24	42664	0.5673
OAS1	10139	6	.	G	.	25.16	RefCall	F	GT:GQ:DP:AF	0/0	20	42277	0.6263

OAS1	10215	6	.	A	.	25.8	RefCall	F	GT:GQ:DP:AF	0/0	26	42530	0.6456
OAS1	10504	6	.	A	.	35.34	RefCall	F	GT:GQ:DP:AF	0/0	29	42742	0.9085
OAS1	11183	6	.	C	.	17.86	RefCall	F	GT:GQ:DP:AF	0/0	24	42783	0.913
OAS1	12119	6	.	C	.	31.94	RefCall	F	GT:GQ:DP:AF	0/0	33	42855	0.9206
OAS1	12975	6	.	A	.	23.01	RefCall	F	GT:GQ:DP:AF	0/0	22	96852	0.7613
OAS1	13092	6	.	C	.	18.14	RefCall	F	GT:GQ:DP:AF	0/0	26	96903	0.6967
OAS1	13100	6	.	A	.	24.38	RefCall	F	GT:GQ:DP:AF	0/0	1	53823	0.5116
OAS1	13891	6	.	A	.	19.43	RefCall	F	GT:GQ:DP:AF	0/0	2	53784	0.4549
OAS1	13931	6	.	A	.	30.64	RefCall	F	GT:GQ:DP:AF	0/0	0	53706	0.2394
OAS1	13958	6	.	G	.	34.29	RefCall	F	GT:GQ:DP:AF	0/0	26	53667	0.8219
OAS1	14108	6	.	C	.	18.08	RefCall	F	GT:GQ:DP:AF	0/0	11	53611	0.3294
OAS1	14345	6	.	C	.	27.31	RefCall	F	GT:GQ:DP:AF	0/0	25	53535	0.7534
OAS1	14359	6	.	G	C	34.42	PASS	F	GT:GQ:DP:AF	1/1	20	52085	0.4663
OAS1	15055	6	.	T	.	20.21	RefCall	F	GT:GQ:DP:AF	0/0	25	53530	0.7441
OAS1	15228	6	.	A	G	32.76	PASS	F	GT:GQ:DP:AF	1/1	32	51728	0.9175
OAS1	45	7	.	C	T	9.54	PASS	F	GT:GQ:DP:AF	0/1	9	22	0.2273
OAS1	50	7	.	A	G	4.55	PASS	F	GT:GQ:DP:AF	0/1	4	25	0.8
OAS1	57	7	.	T	C	7.51	PASS	F	GT:GQ:DP:AF	1/1	7	27	0.7407
OAS1	73	7	.	T	C	6.6	PASS	F	GT:GQ:DP:AF	0/1	6	27	0.4444
OAS1	74	7	.	G	A	5.46	PASS	F	GT:GQ:DP:AF	0/1	5	27	0.2593
OAS1	79	7	.	A	G	7.41	PASS	F	GT:GQ:DP:AF	0/1	7	27	0.2593
OAS1	81	7	.	A	G	4.66	PASS	F	GT:GQ:DP:AF	1/1	4	27	0.7037
OAS1	82	7	.	T	C	7.81	PASS	F	GT:GQ:DP:AF	0/1	7	27	0.2593
OAS1	88	7	.	G	A	8.72	PASS	F	GT:GQ:DP:AF	0/1	8	28	0.5
OAS1	106	7	.	G	A	10.33	PASS	F	GT:GQ:DP:AF	0/1	10	28	0.3214
OAS1	125	7	.	T	.	9.13	RefCall	F	GT:GQ:DP:AF	0/0	11	30	0.4333
OAS1	127	7	.	C	G	2.27	PASS	F	GT:GQ:DP:AF	1/1	9	28	0.6429
OAS1	128	7	.	A	T	1.8	LowQual	F	GT:GQ:DP:AF	1/1	2	28	0.3929
OAS1	136	7	.	C	T	7.06	PASS	F	GT:GQ:DP:AF	0/1	1	28	0.3214
OAS1	137	7	.	A	.	6.8	RefCall	F	GT:GQ:DP:AF	0/0	7	31	0.3871
OAS1	164	7	.	C	T	5.97	PASS	F	GT:GQ:DP:AF	0/1	7	28	0.5714
OAS1	165	7	.	A	.	5.93	RefCall	F	GT:GQ:DP:AF	0/0	4	31	0.7419
OAS1	169	7	.	A	G	8.91	PASS	F	GT:GQ:DP:AF	0/1	6	28	0.3214
OAS1	179	7	.	G	A	6.06	PASS	F	GT:GQ:DP:AF	0/1	5	30	0.2333
OAS1	180	7	.	T	C	5.81	PASS	F	GT:GQ:DP:AF	0/1	5	30	0.6
OAS1	181	7	.	A	G	11.59	PASS	F	GT:GQ:DP:AF	0/1	8	30	0.5
OAS1	214	7	.	G	A	7.38	PASS	F	GT:GQ:DP:AF	0/1	6	30	0.2333
OAS1	225	7	.	C	T	4.39	PASS	F	GT:GQ:DP:AF	1/1	5	30	0.3667
OAS1	241	7	.	A	G	6.04	PASS	F	GT:GQ:DP:AF	0/1	6	31	0.5161
OAS1	257	7	.	A	G	11.21	PASS	F	GT:GQ:DP:AF	0/1	11	30	0.4
OAS1	286	7	.	C	T	9.25	PASS	F	GT:GQ:DP:AF	0/1	30	42206	0.6863

OAS1	287	7	.	A	G	11.15	PASS	F	GT:GQ:DP:AF	0/1	25	41905	0.7228
OAS1	294	7	.	T	C	5.17	PASS	F	GT:GQ:DP:AF	0/1	28	41898	0.5031
OAS1	310	7	.	C	A	6.91	PASS	F	GT:GQ:DP:AF	1/1	23	41754	0.6674
OAS1	315	7	.	T	A	7.29	PASS	F	GT:GQ:DP:AF	1/1	19	41753	0.4092
OAS1	319	7	.	T	A	5.89	PASS	F	GT:GQ:DP:AF	1/1	32	41758	0.8807
OAS1	2210	7	.	T	.	13.33	RefCall	F	GT:GQ:DP:AF	0/0	9	27	0.3704
OAS1	2289	7	.	A	C	7.97	PASS	F	GT:GQ:DP:AF	0/1	32	41758	0.8885
OAS1	2821	7	.	A	C	14.84	PASS	F	GT:GQ:DP:AF	0/1	31	41638	0.9043
OAS1	3411	7	.	A	.	29.41	RefCall	F	GT:GQ:DP:AF	0/0	11	27	0.3704
OAS1	4400	7	.	G	.	27.05	RefCall	F	GT:GQ:DP:AF	0/0	5	27	0.3333
OAS1	4704	7	.	A	.	24.24	RefCall	F	GT:GQ:DP:AF	0/0	6	24	0.5417
OAS1	4750	7	.	G	.	31.46	RefCall	F	GT:GQ:DP:AF	0/0	7	24	0.5
OAS1	5069	7	.	G	.	25.41	RefCall	F	GT:GQ:DP:AF	0/0	5	14	0.6429
OAS1	5188	7	.	G	.	29.51	RefCall	F	GT:GQ:DP:AF	0/0	13	38502	0.5517
OAS1	5331	7	.	T	.	20.29	RefCall	F	GT:GQ:DP:AF	0/0	7	38514	0.4528
OAS1	5395	7	.	C	.	24.56	RefCall	F	GT:GQ:DP:AF	0/0	14	38486	0.4278
OAS1	5543	7	.	G	.	24.29	RefCall	F	GT:GQ:DP:AF	0/0	29	38451	0.7274
OAS1	5971	7	.	C	.	29.32	RefCall	F	GT:GQ:DP:AF	0/0	27	38487	0.8261
OAS1	6220	7	.	T	.	22.33	RefCall	F	GT:GQ:DP:AF	0/0	24	38484	0.6358
OAS1	6305	7	.	G	.	26.31	RefCall	F	GT:GQ:DP:AF	0/0	31	38488	0.8718
OAS1	6955	7	.	A	.	7.68	RefCall	F	GT:GQ:DP:AF	0/0	25	38751	0.878
OAS1	7226	7	.	T	.	32.24	RefCall	F	GT:GQ:DP:AF	0/0	29	38752	0.9165
OAS1	7426	7	.	T	.	15.67	RefCall	F	GT:GQ:DP:AF	0/0	20	38482	0.6232
OAS1	8858	7	.	A	.	22.71	RefCall	F	GT:GQ:DP:AF	0/0	24	38504	0.4671
OAS1	9246	7	.	A	.	19.65	RefCall	F	GT:GQ:DP:AF	0/0	24	38522	0.6891
OAS1	9657	7	.	Gtt	G	1.15	LowQual	F	GT:GQ:DP:AF	1/1	28	41629	0.8024
OAS1	9900	7	.	A	.	15.06	RefCall	F	GT:GQ:DP:AF	0/0	29	38564	0.811
OAS1	10504	7	.	A	.	31.38	RefCall	F	GT:GQ:DP:AF	0/0	22	38609	0.6131
OAS1	11183	7	.	C	.	22.06	RefCall	F	GT:GQ:DP:AF	0/0	26	38645	0.6454
OAS1	11419	7	.	G	.	28.1	RefCall	F	GT:GQ:DP:AF	0/0	7	38767	0.5461
OAS1	11756	7	.	T	.	25	RefCall	F	GT:GQ:DP:AF	0/0	32	38845	0.9131
OAS1	12119	7	.	C	.	30	RefCall	F	GT:GQ:DP:AF	0/0	15	38867	0.3781
OAS1	12975	7	.	A	.	25.25	RefCall	F	GT:GQ:DP:AF	0/0	22	43241	0.5112
OAS1	13100	7	.	A	.	28.23	RefCall	F	GT:GQ:DP:AF	0/0	19	43173	0.457
OAS1	13857	7	.	T	.	23.31	RefCall	F	GT:GQ:DP:AF	0/0	1	43100	0.1233
OAS1	13891	7	.	A	.	19.35	RefCall	F	GT:GQ:DP:AF	0/0	15	43000	0.3274
OAS1	13958	7	.	G	.	32.59	RefCall	F	GT:GQ:DP:AF	0/0	31	42758	0.9003
OAS1	13959	7	.	A	.	32.46	RefCall	F	GT:GQ:DP:AF	0/0	22	42462	0.6186
OAS1	14315	7	.	G	A	31.38	PASS	F	GT:GQ:DP:AF	1/1	32	41620	0.9242
OAS1	14345	7	.	C	.	28.51	RefCall	F	GT:GQ:DP:AF	0/0	28	42441	0.7965
OAS1	14359	7	.	G	C	32.74	PASS	F	GT:GQ:DP:AF	1/1	22	41426	0.4686

OAS1	15055	7	.	T	.	22.91	RefCall	F	GT:GQ:DP:AF	0/0	25	42320	0.6847
OAS1	15228	7	.	A	G	34.71	PASS	F	GT:GQ:DP:AF	1/1	34	41156	0.9226
OAS1	34	8	.	T	A	2.32	PASS	F	GT:GQ:DP:AF	0/1	2	5	0.8
OAS1	50	8	.	A	G	4.43	PASS	F	GT:GQ:DP:AF	1/1	4	21	0.8095
OAS1	81	8	.	A	G	4.94	PASS	F	GT:GQ:DP:AF	1/1	4	23	0.7391
OAS1	82	8	.	T	A	8.72	PASS	F	GT:GQ:DP:AF	0/1	8	23	0.4348
OAS1	88	8	.	G	A	10.21	PASS	F	GT:GQ:DP:AF	0/1	10	24	0.625
OAS1	95	8	.	C	G	9.25	PASS	F	GT:GQ:DP:AF	0/1	9	24	0.2917
OAS1	105	8	.	C	T	11.03	PASS	F	GT:GQ:DP:AF	0/1	11	24	0.4167
OAS1	106	8	.	G	A	7.12	PASS	F	GT:GQ:DP:AF	0/1	7	24	0.25
OAS1	127	8	.	C	G	7.74	PASS	F	GT:GQ:DP:AF	1/1	7	24	0.5
OAS1	128	8	.	A	T	3.2	PASS	F	GT:GQ:DP:AF	1/1	3	24	0.5
OAS1	136	8	.	C	T	8.15	PASS	F	GT:GQ:DP:AF	0/1	8	25	0.4
OAS1	137	8	.	A	.	2.72	RefCall	F	GT:GQ:DP:AF	0/0	4	22	0.5455
OAS1	153	8	.	C	CA	0	LowQual	F	GT:GQ:DP:AF	0/1	2	25	0.56
OAS1	164	8	.	C	T	7.92	PASS	F	GT:GQ:DP:AF	0/1	0	26	0.0769
OAS1	181	8	.	A	G	10.97	PASS	F	GT:GQ:DP:AF	0/1	7	26	0.4231
OAS1	201	8	.	G	A	9.08	PASS	F	GT:GQ:DP:AF	0/1	10	27	0.3333
OAS1	213	8	.	T	.	5.76	RefCall	F	GT:GQ:DP:AF	0/0	4	22	0.3636
OAS1	256	8	.	T	C	6.12	PASS	F	GT:GQ:DP:AF	1/1	9	27	0.2963
OAS1	262	8	.	T	C	4.51	PASS	F	GT:GQ:DP:AF	1/1	5	27	0.7037
OAS1	264	8	.	T	C	4.83	PASS	F	GT:GQ:DP:AF	0/1	6	22	0.6818
OAS1	265	8	.	G	A	9.66	PASS	F	GT:GQ:DP:AF	0/1	9	22	0.3182
OAS1	286	8	.	C	T	9.49	PASS	F	GT:GQ:DP:AF	0/1	3	14	0.5714
OAS1	315	8	.	T	A	3.07	PASS	F	GT:GQ:DP:AF	1/1	18	47118	0.4067
OAS1	319	8	.	T	A	2.68	PASS	F	GT:GQ:DP:AF	0/1	28	47127	0.7584
OAS1	1636	8	.	C	.	20.66	RefCall	F	GT:GQ:DP:AF	0/0	9	19	0.3684
OAS1	2821	8	.	A	C	37.73	PASS	F	GT:GQ:DP:AF	1/1	34	47123	0.8781
OAS1	3368	8	.	C	.	32.42	RefCall	F	GT:GQ:DP:AF	0/0	2	9	0.6667
OAS1	3612	8	.	A	.	28.56	RefCall	F	GT:GQ:DP:AF	0/0	20	37037	0.4639
OAS1	3710	8	.	A	.	18.59	RefCall	F	GT:GQ:DP:AF	0/0	37	37090	0.8887
OAS1	4337	8	.	A	.	27.13	RefCall	F	GT:GQ:DP:AF	0/0	32	37070	0.8788
OAS1	4363	8	.	G	.	24.53	RefCall	F	GT:GQ:DP:AF	0/0	28	37091	0.8033
OAS1	4400	8	.	G	.	29	RefCall	F	GT:GQ:DP:AF	0/0	18	37093	0.5267
OAS1	4704	8	.	A	.	20.03	RefCall	F	GT:GQ:DP:AF	0/0	27	37165	0.9203
OAS1	5188	8	.	G	.	29.16	RefCall	F	GT:GQ:DP:AF	0/0	24	37172	0.8581
OAS1	5303	8	.	G	.	22.3	RefCall	F	GT:GQ:DP:AF	0/0	29	37187	0.8326
OAS1	5331	8	.	T	.	23.08	RefCall	F	GT:GQ:DP:AF	0/0	20	37256	0.642
OAS1	5415	8	.	A	.	27.43	RefCall	F	GT:GQ:DP:AF	0/0	29	37781	0.9168
OAS1	5738	8	.	T	.	28.03	RefCall	F	GT:GQ:DP:AF	0/0	22	37684	0.5662
OAS1	5783	8	.	C	T	28.23	PASS	F	GT:GQ:DP:AF	0/1	31	47125	0.8893

OAS1	5992	8	.	G	A	30.11	PASS	F	GT:GQ:DP:AF	1/1	6	47103	0.6394
OAS1	6042	8	.	C	.	30.37	RefCall	F	GT:GQ:DP:AF	0/0	23	37399	0.6311
OAS1	6215	8	.	A	.	26.3	RefCall	F	GT:GQ:DP:AF	0/0	27	37423	0.7348
OAS1	6220	8	.	T	.	18.59	RefCall	F	GT:GQ:DP:AF	0/0	28	37479	0.7835
OAS1	6258	8	.	A	.	20.96	RefCall	F	GT:GQ:DP:AF	0/0	28	37476	0.5565
OAS1	6915	8	.	C	.	22.74	RefCall	F	GT:GQ:DP:AF	0/0	30	37512	0.9258
OAS1	6955	8	.	A	G	29.25	PASS	F	GT:GQ:DP:AF	0/1	36	47058	0.9061
OAS1	7068	8	.	T	.	26.53	RefCall	F	GT:GQ:DP:AF	0/0	30	37536	0.8375
OAS1	7101	8	.	C	.	33.99	RefCall	F	GT:GQ:DP:AF	0/0	26	37594	0.7707
OAS1	7426	8	.	T	.	15.96	RefCall	F	GT:GQ:DP:AF	0/0	18	37593	0.6096
OAS1	7983	8	.	C	.	23.55	RefCall	F	GT:GQ:DP:AF	0/0	20	37610	0.6061
OAS1	8580	8	.	A	.	28.02	RefCall	F	GT:GQ:DP:AF	0/0	22	37776	0.6675
OAS1	8642	8	.	T	G	38.77	PASS	F	GT:GQ:DP:AF	1/1	26	47064	0.8026
OAS1	8858	8	.	A	.	16.65	RefCall	F	GT:GQ:DP:AF	0/0	29	37790	0.5435
OAS1	9657	8	.	Gtttt	G	0	LowQual	F	GT:GQ:DP:AF	0/1	36	47057	0.9203
OAS1	9900	8	.	A	.	1.61	RefCall	F	GT:GQ:DP:AF	0/0	26	37835	0.9108
OAS1	9991	8	.	G	.	28.97	RefCall	F	GT:GQ:DP:AF	0/0	33	37854	0.8077
OAS1	10504	8	.	A	.	36.25	RefCall	F	GT:GQ:DP:AF	0/0	15	37892	0.3798
OAS1	11183	8	.	C	.	16.57	RefCall	F	GT:GQ:DP:AF	0/0	23	86099	0.7627
OAS1	11419	8	.	G	.	26.68	RefCall	F	GT:GQ:DP:AF	0/0	28	86173	0.6977
OAS1	12119	8	.	C	.	24.21	RefCall	F	GT:GQ:DP:AF	0/0	38	86155	0.8991
OAS1	13100	8	.	A	.	24.96	RefCall	F	GT:GQ:DP:AF	0/0	16	48224	0.5143
OAS1	13891	8	.	A	.	18.79	RefCall	F	GT:GQ:DP:AF	0/0	0	48003	0.1302
OAS1	13931	8	.	A	.	28.68	RefCall	F	GT:GQ:DP:AF	0/0	1	47887	0.3276
OAS1	13958	8	.	G	.	34.48	RefCall	F	GT:GQ:DP:AF	0/0	28	47848	0.9183
OAS1	13959	8	.	A	.	31.06	RefCall	F	GT:GQ:DP:AF	0/0	36	47644	0.8998
OAS1	14108	8	.	C	.	6.58	RefCall	F	GT:GQ:DP:AF	0/0	16	47543	0.621
OAS1	14315	8	.	G	A	36.43	PASS	F	GT:GQ:DP:AF	1/1	16	46989	0.4642
OAS1	14345	8	.	C	.	26.33	RefCall	F	GT:GQ:DP:AF	0/0	26	47505	0.7871
OAS1	14359	8	.	G	C	36.75	PASS	F	GT:GQ:DP:AF	1/1	26	46905	0.7419
OAS1	15055	8	.	T	.	16.91	RefCall	F	GT:GQ:DP:AF	0/0	24	47277	0.6819
OAS1	15178	8	.	G	.	26.56	RefCall	F	GT:GQ:DP:AF	0/0	24	47216	0.5038
OAS1	15228	8	.	A	G	34.62	PASS	F	GT:GQ:DP:AF	1/1	34	46676	0.9172
OAS1	50	9	.	A	G	6.82	PASS	F	GT:GQ:DP:AF	1/1	6	24	0.875
OAS1	57	9	.	T	C	6.32	PASS	F	GT:GQ:DP:AF	1/1	6	26	0.8077
OAS1	73	9	.	T	C	5.6	PASS	F	GT:GQ:DP:AF	0/1	5	28	0.4643
OAS1	74	9	.	G	A	8.91	PASS	F	GT:GQ:DP:AF	0/1	8	28	0.3214
OAS1	81	9	.	A	G	10.64	PASS	F	GT:GQ:DP:AF	1/1	10	28	0.8929
OAS1	88	9	.	G	A	10.39	PASS	F	GT:GQ:DP:AF	0/1	10	29	0.4483
OAS1	96	9	.	C	T	9.79	PASS	F	GT:GQ:DP:AF	0/1	9	29	0.5517
OAS1	105	9	.	C	T	9.37	PASS	F	GT:GQ:DP:AF	0/1	9	29	0.3103

OAS1	106	9	.	G	A	12.49	PASS	F	GT:GQ:DP:AF	0/1	12	29	0.4483
OAS1	126	9	.	G	.	5.6	RefCall	F	GT:GQ:DP:AF	0/0	8	25	0.28
OAS1	128	9	.	A	T	3.25	PASS	F	GT:GQ:DP:AF	1/1	5	29	0.7586
OAS1	180	9	.	T	C	3.12	PASS	F	GT:GQ:DP:AF	1/1	3	29	0.4483
OAS1	181	9	.	A	G	10.19	PASS	F	GT:GQ:DP:AF	0/1	3	30	0.3667
OAS1	202	9	.	G	.	6.17	RefCall	F	GT:GQ:DP:AF	0/0	7	23	0.3043
OAS1	219	9	.	G	A	8.38	PASS	F	GT:GQ:DP:AF	0/1	10	30	0.3333
OAS1	223	9	.	A	G	10.04	PASS	F	GT:GQ:DP:AF	0/1	6	30	0.8333
OAS1	225	9	.	C	T	4.18	PASS	F	GT:GQ:DP:AF	0/1	8	28	0.3929
OAS1	241	9	.	A	.	6.87	RefCall	F	GT:GQ:DP:AF	0/0	5	23	0.3043
OAS1	256	9	.	T	C	4.93	PASS	F	GT:GQ:DP:AF	0/1	10	28	0.5
OAS1	258	9	.	A	T	13.33	PASS	F	GT:GQ:DP:AF	0/1	4	28	0.7857
OAS1	262	9	.	T	C	9.77	PASS	F	GT:GQ:DP:AF	0/1	6	27	0.3333
OAS1	264	9	.	T	C	7.06	PASS	F	GT:GQ:DP:AF	0/1	4	27	0.5185
OAS1	287	9	.	A	G	8.52	PASS	F	GT:GQ:DP:AF	0/1	13	27	0.3704
OAS1	294	9	.	T	C	7.55	PASS	F	GT:GQ:DP:AF	0/1	9	27	0.4444
OAS1	295	9	.	G	A	5.66	PASS	F	GT:GQ:DP:AF	0/1	7	27	0.5556
OAS1	310	9	.	C	A	7.78	PASS	F	GT:GQ:DP:AF	1/1	7	18	0.3889
OAS1	323	9	.	T	.	14.52	RefCall	F	GT:GQ:DP:AF	0/0	14	7	0.5714
OAS1	325	9	.	T	.	9.54	RefCall	F	GT:GQ:DP:AF	0/0	9	5	0.6
OAS1	331	9	.	G	.	11.52	RefCall	F	GT:GQ:DP:AF	0/0	11	3	0.3333
OAS1	1636	9	.	C	.	17.95	RefCall	F	GT:GQ:DP:AF	0/0	17	39386	0.4639
OAS1	2748	9	.	C	.	29.19	RefCall	F	GT:GQ:DP:AF	0/0	29	39442	0.7399
OAS1	3189	9	.	G	.	27.21	RefCall	F	GT:GQ:DP:AF	0/0	27	39462	0.7544
OAS1	3411	9	.	A	.	31.71	RefCall	F	GT:GQ:DP:AF	0/0	31	39442	0.7272
OAS1	3612	9	.	A	.	29.81	RefCall	F	GT:GQ:DP:AF	0/0	29	39417	0.8025
OAS1	4400	9	.	G	.	29.98	RefCall	F	GT:GQ:DP:AF	0/0	29	39501	0.8264
OAS1	4704	9	.	A	.	18.12	RefCall	F	GT:GQ:DP:AF	0/0	18	39509	0.6342
OAS1	4750	9	.	G	.	34.72	RefCall	F	GT:GQ:DP:AF	0/0	34	39495	0.8714
OAS1	5188	9	.	G	.	26.56	RefCall	F	GT:GQ:DP:AF	0/0	26	40116	0.9145
OAS1	5295	9	.	G	.	20.46	RefCall	F	GT:GQ:DP:AF	0/0	20	40058	0.6156
OAS1	5331	9	.	T	.	17.92	RefCall	F	GT:GQ:DP:AF	0/0	17	39586	0.6322
OAS1	5381	9	.	T	.	28.37	RefCall	F	GT:GQ:DP:AF	0/0	28	39577	0.7649
OAS1	5395	9	.	C	.	18.11	RefCall	F	GT:GQ:DP:AF	0/0	18	39569	0.4674
OAS1	5776	9	.	C	.	28.31	RefCall	F	GT:GQ:DP:AF	0/0	28	39564	0.6887
OAS1	5971	9	.	C	.	32.57	RefCall	F	GT:GQ:DP:AF	0/0	32	39587	0.8122
OAS1	6215	9	.	A	.	19.56	RefCall	F	GT:GQ:DP:AF	0/0	19	39612	0.7665
OAS1	6220	9	.	T	.	21.54	RefCall	F	GT:GQ:DP:AF	0/0	21	39610	0.6123
OAS1	6305	9	.	G	.	21.35	RefCall	F	GT:GQ:DP:AF	0/0	21	39650	0.6508
OAS1	6996	9	.	A	.	31.46	RefCall	F	GT:GQ:DP:AF	0/0	31	39779	0.9059
OAS1	7068	9	.	T	.	24.65	RefCall	F	GT:GQ:DP:AF	0/0	24	39800	0.9141

OAS1	7101	9	.	C	.	35.61	RefCall	F	GT:GQ:DP:AF	0/0	35	39811	0.8126
OAS1	7227	9	.	A	G	27.57	PASS	F	GT:GQ:DP:AF	0/1	30	41667	0.4839
OAS1	7426	9	.	Taa	T	2.08	PASS	F	GT:GQ:DP:AF	0/1	29	41590	0.7921
OAS1	7983	9	.	C	.	25.8	RefCall	F	GT:GQ:DP:AF	0/0	27	39852	0.4856
OAS1	8858	9	.	A	.	9.57	RefCall	F	GT:GQ:DP:AF	0/0	2	39915	0.1695
OAS1	9281	9	.	T	C	32.58	PASS	F	GT:GQ:DP:AF	1/1	30	41544	0.8895
OAS1	9285	9	.	C	.	31.07	RefCall	F	GT:GQ:DP:AF	0/0	25	82833	0.7626
OAS1	9657	9	.	Gttt	G,Gt	0	LowQual	F	GT:GQ:DP:AF	1/2	2	41525	0.6323
OAS1	9830	9	.	C	A	24.91	PASS	F	GT:GQ:DP:AF	0/1	33	41494	0.9064
OAS1	9900	9	.	A	.	4.64	RefCall	F	GT:GQ:DP:AF	0/0	9	42738	0.3908
OAS1	10225	9	.	A	.	10.87	RefCall	F	GT:GQ:DP:AF	0/0	32	42722	0.863
OAS1	10504	9	.	A	.	35.95	RefCall	F	GT:GQ:DP:AF	0/0	31	42724	0.8613
OAS1	10628	9	.	G	.	25.21	RefCall	F	GT:GQ:DP:AF	0/0	0	42670	0.2465
OAS1	11183	9	.	C	.	19.34	RefCall	F	GT:GQ:DP:AF	0/0	24	42627	0.4649
OAS1	12119	9	.	C	.	31.25	RefCall	F	GT:GQ:DP:AF	0/0	4	42597	0.3258
OAS1	13092	9	.	C	.	21.59	RefCall	F	GT:GQ:DP:AF	0/0	10	42487	0.7097
OAS1	13100	9	.	A	.	23.2	RefCall	F	GT:GQ:DP:AF	0/0	35	42409	0.9033
OAS1	13157	9	.	A	G	30.06	PASS	F	GT:GQ:DP:AF	0/1	34	41491	0.9134
OAS1	13688	9	.	C	.	29.83	RefCall	F	GT:GQ:DP:AF	0/0	25	42357	0.7304
OAS1	13959	9	.	A	.	30.07	RefCall	F	GT:GQ:DP:AF	0/0	19	42134	0.6244
OAS1	14108	9	.	C	.	2.4	RefCall	F	GT:GQ:DP:AF	0/0	31	41943	0.6863
OAS1	14315	9	.	G	A	33.15	PASS	F	GT:GQ:DP:AF	1/1	36	41477	0.9236
OAS1	14331	9	.	G	A	34.44	PASS	F	GT:GQ:DP:AF	1/1	24	41326	0.5991
OAS1	14359	9	.	G	C	36.25	PASS	F	GT:GQ:DP:AF	1/1	8	41323	0.4652
OAS1	15045	9	.	A	.	24.69	RefCall	F	GT:GQ:DP:AF	0/0	21	41682	0.5215
OAS1	15055	9	.	T	.	8.98	RefCall	F	GT:GQ:DP:AF	0/0	23	41685	0.5044
OAS1	15228	9	.	A	G	31.64	PASS	F	GT:GQ:DP:AF	1/1	31	41014	0.921
OAS1	34	10	.	T	.	10.85	RefCall	F	GT:GQ:DP:AF	0/0	5	12	0.5833
OAS1	43	10	.	T	C	2.69	PASS	F	GT:GQ:DP:AF	1/1	10	2	0
OAS1	50	10	.	A	G	2.94	PASS	F	GT:GQ:DP:AF	1/1	2	6	1
OAS1	57	10	.	T	.	3.93	RefCall	F	GT:GQ:DP:AF	0/0	7	12	0.3333
OAS1	81	10	.	A	.	3.04	RefCall	F	GT:GQ:DP:AF	0/0	9	11	0.7273
OAS1	82	10	.	T	.	5.96	RefCall	F	GT:GQ:DP:AF	0/0	5	11	0.4545
OAS1	87	10	.	C	T	4.96	PASS	F	GT:GQ:DP:AF	0/1	2	8	0.875
OAS1	88	10	.	G	.	6.72	RefCall	F	GT:GQ:DP:AF	0/0	6	11	0.3636
OAS1	95	10	.	C	G	3.77	PASS	F	GT:GQ:DP:AF	0/1	3	10	0.3
OAS1	96	10	.	C	T	6.84	PASS	F	GT:GQ:DP:AF	0/1	3	11	0.2727
OAS1	105	10	.	C	T	6.71	PASS	F	GT:GQ:DP:AF	0/1	5	11	0.5455
OAS1	114	10	.	A	C	2.81	PASS	F	GT:GQ:DP:AF	0/1	4	11	0.2727
OAS1	121	10	.	C	A	6.11	PASS	F	GT:GQ:DP:AF	1/1	6	11	0.4545
OAS1	127	10	.	C	G	5.39	PASS	F	GT:GQ:DP:AF	1/1	3	11	0.5455

OAS1	128	10	.	A	.	4.33	RefCall	F	GT:GQ:DP:AF	0/0	6	11	0.6364
OAS1	137	10	.	A	.	8.77	RefCall	F	GT:GQ:DP:AF	0/0	3	11	0.7273
OAS1	145	10	.	A	.	7.29	RefCall	F	GT:GQ:DP:AF	0/0	3	11	0.4545
OAS1	153	10	.	C	.	3.83	RefCall	F	GT:GQ:DP:AF	0/0	5	11	0.5455
OAS1	169	10	.	A	G	7.76	PASS	F	GT:GQ:DP:AF	0/1	6	11	0.7273
OAS1	178	10	.	T	.	6.74	RefCall	F	GT:GQ:DP:AF	0/0	3	11	0.6364
OAS1	179	10	.	G	.	5.72	RefCall	F	GT:GQ:DP:AF	0/0	6	11	0.4545
OAS1	180	10	.	T	C	2.83	PASS	F	GT:GQ:DP:AF	1/1	6	11	0.3636
OAS1	200	10	.	T	C	7.65	PASS	F	GT:GQ:DP:AF	0/1	2	11	0.7273
OAS1	213	10	.	T	.	9.39	RefCall	F	GT:GQ:DP:AF	0/0	8	11	0.7273
OAS1	214	10	.	G	A	5.96	PASS	F	GT:GQ:DP:AF	0/1	6	11	0.7273
OAS1	219	10	.	G	A	6.44	PASS	F	GT:GQ:DP:AF	0/1	5	11	0.7273
OAS1	223	10	.	A	G	6.18	PASS	F	GT:GQ:DP:AF	0/1	4	11	0.0909
OAS1	225	10	.	C	T	3.13	PASS	F	GT:GQ:DP:AF	0/1	8	12	0.5
OAS1	241	10	.	A	G	3.77	PASS	F	GT:GQ:DP:AF	1/1	7	12	0.6667
OAS1	256	10	.	T	C	5.67	PASS	F	GT:GQ:DP:AF	1/1	3	12	0.5
OAS1	262	10	.	T	C	3.92	PASS	F	GT:GQ:DP:AF	1/1	7	12	0.4167
OAS1	264	10	.	T	C	6.4	PASS	F	GT:GQ:DP:AF	0/1	6	12	0.6667
OAS1	276	10	.	C	.	8.53	RefCall	F	GT:GQ:DP:AF	0/0	31	39778	0.8074
OAS1	1636	10	.	C	.	21.44	RefCall	F	GT:GQ:DP:AF	0/0	30	39800	0.7268
OAS1	2378	10	.	C	G	31.82	PASS	F	GT:GQ:DP:AF	1/1	2	12	0.4167
OAS1	3411	10	.	A	.	30.7	RefCall	F	GT:GQ:DP:AF	0/0	26	39776	0.8006
OAS1	3612	10	.	A	.	26.7	RefCall	F	GT:GQ:DP:AF	0/0	23	39771	0.5271
OAS1	3820	10	.	C	.	23.94	RefCall	F	GT:GQ:DP:AF	0/0	30	39808	0.8263
OAS1	4400	10	.	G	.	30.52	RefCall	F	GT:GQ:DP:AF	0/0	31	39831	0.8465
OAS1	4678	10	.	A	.	31.34	RefCall	F	GT:GQ:DP:AF	0/0	29	39857	0.8148
OAS1	5005	10	.	A	.	29.22	RefCall	F	GT:GQ:DP:AF	0/0	27	40104	0.8756
OAS1	5069	10	.	G	.	27.63	RefCall	F	GT:GQ:DP:AF	0/0	25	40105	0.9118
OAS1	5188	10	.	G	.	25.3	RefCall	F	GT:GQ:DP:AF	0/0	27	39890	0.7664
OAS1	5381	10	.	T	.	27.3	RefCall	F	GT:GQ:DP:AF	0/0	12	39904	0.4692
OAS1	5395	10	.	C	.	12.86	RefCall	F	GT:GQ:DP:AF	0/0	28	39912	0.7359
OAS1	5415	10	.	A	.	28.24	RefCall	F	GT:GQ:DP:AF	0/0	26	39878	0.6869
OAS1	5776	10	.	C	.	26.57	RefCall	F	GT:GQ:DP:AF	0/0	30	39888	0.8075
OAS1	5971	10	.	C	.	30.67	RefCall	F	GT:GQ:DP:AF	0/0	20	39894	0.7701
OAS1	6215	10	.	A	.	20.55	RefCall	F	GT:GQ:DP:AF	0/0	21	39901	0.6454
OAS1	6305	10	.	G	.	21.2	RefCall	F	GT:GQ:DP:AF	0/0	20	39992	0.6711
OAS1	6915	10	.	C	.	20.03	RefCall	F	GT:GQ:DP:AF	0/0	32	39999	0.9057
OAS1	6996	10	.	A	.	32.31	RefCall	F	GT:GQ:DP:AF	0/0	27	40021	0.9127
OAS1	7068	10	.	T	.	27.07	RefCall	F	GT:GQ:DP:AF	0/0	27	40088	0.4377
OAS1	7227	10	.	A	G	27.55	PASS	F	GT:GQ:DP:AF	0/1	21	39726	0.4692
OAS1	7695	10	.	C	.	24.6	RefCall	F	GT:GQ:DP:AF	0/0	24	91936	0.6909

OAS1	7983	10	.	C	.	24.36	RefCall	F	GT:GQ:DP:AF	0/0	24	92174	0.7625
OAS1	8642	10	.	T	G	38.66	PASS	F	GT:GQ:DP:AF	1/1	38	92311	0.8993
OAS1	8651	10	.	G	.	31.75	RefCall	F	GT:GQ:DP:AF	0/0	31	92309	0.8822
OAS1	9246	10	.	A	.	11.75	RefCall	F	GT:GQ:DP:AF	0/0	11	51625	0.453
OAS1	9657	10	.	Gttt	G	0	LowQual	F	GT:GQ:DP:AF	0/1	35	49897	0.9042
OAS1	9830	10	.	C	A	26.23	PASS	F	GT:GQ:DP:AF	0/1	34	49883	0.9234
OAS1	9900	10	.	A	.	6.59	RefCall	F	GT:GQ:DP:AF	0/0	0	51542	0.1316
OAS1	9991	10	.	G	.	28.07	RefCall	F	GT:GQ:DP:AF	0/0	26	51481	0.452
OAS1	10139	10	.	G	.	26.28	RefCall	F	GT:GQ:DP:AF	0/0	6	51474	0.3297
OAS1	10225	10	.	A	.	17.64	RefCall	F	GT:GQ:DP:AF	0/0	28	51455	0.9172
OAS1	11183	10	.	C	.	15.61	RefCall	F	GT:GQ:DP:AF	0/0	26	51373	0.753
OAS1	12119	10	.	C	.	27.79	RefCall	F	GT:GQ:DP:AF	0/0	17	51356	0.709
OAS1	12894	10	.	G	.	30.6	RefCall	F	GT:GQ:DP:AF	0/0	15	50945	0.6199
OAS1	12975	10	.	A	.	22.58	RefCall	F	GT:GQ:DP:AF	0/0	27	50464	0.6818
OAS1	13092	10	.	C	.	20.81	RefCall	F	GT:GQ:DP:AF	0/0	30	50196	0.7912
OAS1	13100	10	.	A	.	24.04	RefCall	F	GT:GQ:DP:AF	0/0	22	50219	0.7247
OAS1	13931	10	.	A	.	26.88	RefCall	F	GT:GQ:DP:AF	0/0	20	50213	0.5205
OAS1	13958	10	.	G	.	34.42	RefCall	F	GT:GQ:DP:AF	0/0	24	50216	0.5003
OAS1	13959	10	.	A	.	32.32	RefCall	F	GT:GQ:DP:AF	0/0	26	49987	0.7608
OAS1	14108	10	.	C	.	16.75	RefCall	F	GT:GQ:DP:AF	0/0	34	49988	0.8789
OAS1	14315	10	.	G	A	35.07	PASS	F	GT:GQ:DP:AF	1/1	25	49879	0.7302
OAS1	14359	10	.	G	C	34.87	PASS	F	GT:GQ:DP:AF	1/1	20	49783	0.4648
OAS1	14495	10	.	T	.	25.36	RefCall	F	GT:GQ:DP:AF	0/0	32	49990	0.8902
OAS1	15055	10	.	T	.	20.69	RefCall	F	GT:GQ:DP:AF	0/0	16	49942	0.6426
OAS1	15228	10	.	A	G	35.5	PASS	F	GT:GQ:DP:AF	1/1	35	49417	0.9253
OAS1	34	11	.	T	.	4.16	RefCall	F	GT:GQ:DP:AF	0/0	5	17	0.6471
OAS1	43	11	.	T	C	3.58	PASS	F	GT:GQ:DP:AF	0/1	4	6	0.1667
OAS1	46	11	.	G	A	7.65	PASS	F	GT:GQ:DP:AF	0/1	3	15	0.8667
OAS1	73	11	.	T	C	6.47	PASS	F	GT:GQ:DP:AF	0/1	7	15	0.2
OAS1	81	11	.	A	G	7	PASS	F	GT:GQ:DP:AF	1/1	6	16	0.375
OAS1	88	11	.	G	A	7.02	PASS	F	GT:GQ:DP:AF	0/1	7	16	0.75
OAS1	128	11	.	A	T	0	LowQual	F	GT:GQ:DP:AF	1/1	7	16	0.3125
OAS1	133	11	.	C	.	5.98	RefCall	F	GT:GQ:DP:AF	0/0	6	9	0.6667
OAS1	136	11	.	C	.	4.7	RefCall	F	GT:GQ:DP:AF	0/0	8	9	0.5556
OAS1	145	11	.	A	T	9.14	PASS	F	GT:GQ:DP:AF	0/1	0	16	0.3125
OAS1	159	11	.	T	C	9.04	PASS	F	GT:GQ:DP:AF	0/1	5	16	0.5
OAS1	201	11	.	G	C	6.97	PASS	F	GT:GQ:DP:AF	0/1	4	16	0.5
OAS1	225	11	.	C	T	9.1	PASS	F	GT:GQ:DP:AF	1/1	9	16	0.5625
OAS1	241	11	.	A	.	5.02	RefCall	F	GT:GQ:DP:AF	0/0	5	8	0.375
OAS1	262	11	.	T	C	7.11	PASS	F	GT:GQ:DP:AF	0/1	9	16	0.3125
OAS1	264	11	.	T	C	5.96	PASS	F	GT:GQ:DP:AF	0/1	6	17	0.2353

OAS1	287	11	.	A	G	6.71	PASS	F	GT:GQ:DP:AF	0/1	9	17	0.8824
OAS1	294	11	.	T	.	8.98	RefCall	F	GT:GQ:DP:AF	0/0	4	6	0.5
OAS1	309	11	.	A	.	7.2	RefCall	F	GT:GQ:DP:AF	0/0	8	4	0.5
OAS1	310	11	.	C	A	5.72	PASS	F	GT:GQ:DP:AF	0/1	5	17	0.2353
OAS1	315	11	.	T	.	4.31	RefCall	F	GT:GQ:DP:AF	0/0	19	41448	0.4664
OAS1	319	11	.	T	.	8.08	RefCall	F	GT:GQ:DP:AF	0/0	3	41474	0.5593
OAS1	1636	11	.	C	.	19.51	RefCall	F	GT:GQ:DP:AF	0/0	17	41476	0.2542
OAS1	2210	11	.	T	.	3.47	RefCall	F	GT:GQ:DP:AF	0/0	19	41490	0.7156
OAS1	2373	11	.	T	.	17.94	RefCall	F	GT:GQ:DP:AF	0/0	31	41411	0.7266
OAS1	2823	11	.	C	.	19.58	RefCall	F	GT:GQ:DP:AF	0/0	29	41399	0.7975
OAS1	3411	11	.	A	.	31.6	RefCall	F	GT:GQ:DP:AF	0/0	28	41393	0.8845
OAS1	3612	11	.	A	.	29.16	RefCall	F	GT:GQ:DP:AF	0/0	24	41389	0.8583
OAS1	4344	11	.	T	.	28.69	RefCall	F	GT:GQ:DP:AF	0/0	25	41406	0.8235
OAS1	4363	11	.	G	.	24.21	RefCall	F	GT:GQ:DP:AF	0/0	24	41456	0.6387
OAS1	4400	11	.	G	.	25.09	RefCall	F	GT:GQ:DP:AF	0/0	32	41462	0.8679
OAS1	4704	11	.	A	.	24.9	RefCall	F	GT:GQ:DP:AF	0/0	29	41714	0.9166
OAS1	4886	11	.	G	.	32.37	RefCall	F	GT:GQ:DP:AF	0/0	21	41695	0.6186
OAS1	5188	11	.	G	.	29.24	RefCall	F	GT:GQ:DP:AF	0/0	15	41562	0.6291
OAS1	5295	11	.	G	.	21.22	RefCall	F	GT:GQ:DP:AF	0/0	25	41574	0.7618
OAS1	5331	11	.	T	.	15.06	RefCall	F	GT:GQ:DP:AF	0/0	19	41570	0.4628
OAS1	5381	11	.	T	.	25.93	RefCall	F	GT:GQ:DP:AF	0/0	28	41631	0.7834
OAS1	5395	11	.	C	.	19.88	RefCall	F	GT:GQ:DP:AF	0/0	32	41625	0.8256
OAS1	5738	11	.	T	.	28.62	RefCall	F	GT:GQ:DP:AF	0/0	18	41666	0.6116
OAS1	5971	11	.	C	.	32.28	RefCall	F	GT:GQ:DP:AF	0/0	32	41819	0.9091
OAS1	6220	11	.	T	.	18.91	RefCall	F	GT:GQ:DP:AF	0/0	27	41839	0.9119
OAS1	6996	11	.	A	.	32.77	RefCall	F	GT:GQ:DP:AF	0/0	36	41846	0.8094
OAS1	7068	11	.	T	.	27.38	RefCall	F	GT:GQ:DP:AF	0/0	30	41872	0.9101
OAS1	7101	11	.	C	.	36.74	RefCall	F	GT:GQ:DP:AF	0/0	28	57669	0.8024
OAS1	7226	11	.	T	.	30.42	RefCall	F	GT:GQ:DP:AF	0/0	5	57572	0.4652
OAS1	8898	11	.	T	.	28.07	RefCall	F	GT:GQ:DP:AF	0/0	0	57473	0.1222
OAS1	9246	11	.	At	A	5.43	PASS	F	GT:GQ:DP:AF	0/1	7	17	0.7059
OAS1	9657	11	.	Gtt	G	0	LowQual	F	GT:GQ:DP:AF	1/1	7	8	0.625
OAS1	9900	11	.	A	.	9.15	RefCall	F	GT:GQ:DP:AF	0/0	31	57447	0.9249
OAS1	9915	11	.	C	.	31.25	RefCall	F	GT:GQ:DP:AF	0/0	35	57195	0.9015
OAS1	10504	11	.	A	.	35.97	RefCall	F	GT:GQ:DP:AF	0/0	13	56801	0.6217
OAS1	11183	11	.	C	.	13.93	RefCall	F	GT:GQ:DP:AF	0/0	30	56723	0.7852
OAS1	11387	11	.	C	.	30.68	RefCall	F	GT:GQ:DP:AF	0/0	26	56710	0.7864
OAS1	11419	11	.	G	.	26.04	RefCall	F	GT:GQ:DP:AF	0/0	26	56365	0.4758
OAS1	12479	11	.	T	G	26.61	PASS	F	GT:GQ:DP:AF	0/1	9	57446	0.3284
OAS1	12894	11	.	G	.	30.81	RefCall	F	GT:GQ:DP:AF	0/0	30	56278	0.7882
OAS1	13092	11	.	C	.	19.06	RefCall	F	GT:GQ:DP:AF	0/0	19	56226	0.513

OAS1	13100	11	.	A	.	18.85	RefCall	F	GT:GQ:DP:AF	0/0	18	56226	0.5025
OAS1	13688	11	.	C	.	29.99	RefCall	F	GT:GQ:DP:AF	0/0	29	56090	0.7969
OAS1	13931	11	.	A	.	27.96	RefCall	F	GT:GQ:DP:AF	0/0	27	56056	0.7614
OAS1	14108	11	.	C	.	15.64	RefCall	F	GT:GQ:DP:AF	0/0	15	56046	0.6396
OAS1	14345	11	.	C	.	32.86	RefCall	F	GT:GQ:DP:AF	0/0	32	55993	0.7871
OAS1	15045	11	.	A	.	20.6	RefCall	F	GT:GQ:DP:AF	0/0	20	55780	0.5993
OAS1	15055	11	.	T	.	21.23	RefCall	F	GT:GQ:DP:AF	0/0	21	55778	0.4617
OAS1	34	12	.	T	.	7.66	RefCall	F	GT:GQ:DP:AF	0/0	18	39744	0.4702
OAS1	43	12	.	T	C	16.64	PASS	F	GT:GQ:DP:AF	1/1	7	15	0.1333
OAS1	46	12	.	G	A	8.61	PASS	F	GT:GQ:DP:AF	0/1	16	25	0.84
OAS1	50	12	.	A	G	6.49	PASS	F	GT:GQ:DP:AF	1/1	8	26	0.2308
OAS1	73	12	.	T	C	6.33	PASS	F	GT:GQ:DP:AF	0/1	6	27	0.8148
OAS1	81	12	.	A	G	6.81	PASS	F	GT:GQ:DP:AF	1/1	6	31	0.5161
OAS1	106	12	.	G	A	5.13	PASS	F	GT:GQ:DP:AF	0/1	6	31	0.7097
OAS1	128	12	.	A	T	4.8	PASS	F	GT:GQ:DP:AF	1/1	5	31	0.4516
OAS1	137	12	.	A	G	5.89	PASS	F	GT:GQ:DP:AF	0/1	4	31	0.5806
OAS1	200	12	.	T	C	5.9	PASS	F	GT:GQ:DP:AF	0/1	5	31	0.3548
OAS1	201	12	.	G	A	6.23	PASS	F	GT:GQ:DP:AF	0/1	5	32	0.3125
OAS1	213	12	.	T	.	6.83	RefCall	F	GT:GQ:DP:AF	0/0	16	39735	0.5544
OAS1	214	12	.	G	A	8.12	PASS	F	GT:GQ:DP:AF	0/1	6	32	0.2188
OAS1	223	12	.	A	G	5.77	PASS	F	GT:GQ:DP:AF	0/1	6	32	0.6875
OAS1	256	12	.	T	C	5.84	PASS	F	GT:GQ:DP:AF	1/1	8	32	0.3438
OAS1	257	12	.	A	G	5.32	PASS	F	GT:GQ:DP:AF	0/1	5	32	0.4375
OAS1	262	12	.	T	C	3.13	PASS	F	GT:GQ:DP:AF	1/1	5	30	0.5333
OAS1	264	12	.	T	C	5.07	PASS	F	GT:GQ:DP:AF	0/1	5	29	0.5172
OAS1	265	12	.	G	A	11.42	PASS	F	GT:GQ:DP:AF	0/1	3	28	0.4286
OAS1	286	12	.	C	T	5.32	PASS	F	GT:GQ:DP:AF	0/1	5	28	0.4286
OAS1	300	12	.	C	T	5.89	PASS	F	GT:GQ:DP:AF	0/1	11	28	0.4643
OAS1	310	12	.	C	A	6.26	PASS	F	GT:GQ:DP:AF	1/1	5	28	0.3571
OAS1	315	12	.	T	A	4.66	PASS	F	GT:GQ:DP:AF	1/1	5	26	0.3846
OAS1	1268	12	.	C	G	12.14	PASS	F	GT:GQ:DP:AF	0/1	6	21	0.4762
OAS1	1636	12	.	C	.	18.6	RefCall	F	GT:GQ:DP:AF	0/0	20	39741	0.5248
OAS1	2210	12	.	T	.	16.16	RefCall	F	GT:GQ:DP:AF	0/0	25	39724	0.7078
OAS1	2373	12	.	T	.	20.23	RefCall	F	GT:GQ:DP:AF	0/0	23	39671	0.754
OAS1	2816	12	.	C	.	25.76	RefCall	F	GT:GQ:DP:AF	0/0	28	39625	0.7328
OAS1	3189	12	.	G	.	23.3	RefCall	F	GT:GQ:DP:AF	0/0	23	39590	0.8025
OAS1	3411	12	.	A	.	28.19	RefCall	F	GT:GQ:DP:AF	0/0	22	39610	0.5241
OAS1	3612	12	.	A	.	23.1	RefCall	F	GT:GQ:DP:AF	0/0	29	39664	0.885
OAS1	3710	12	.	A	.	22.43	RefCall	F	GT:GQ:DP:AF	0/0	26	39656	0.8212
OAS1	4201	12	.	T	.	29.84	RefCall	F	GT:GQ:DP:AF	0/0	25	39656	0.6364
OAS1	4400	12	.	G	.	26.9	RefCall	F	GT:GQ:DP:AF	0/0	28	39653	0.8885

OAS1	4704	12	.	A	.	25.87	RefCall	F	GT:GQ:DP:AF	0/0	25	39672	0.8148
OAS1	4741	12	.	C	.	28.17	RefCall	F	GT:GQ:DP:AF	0/0	24	40057	0.9157
OAS1	5005	12	.	A	.	25.72	RefCall	F	GT:GQ:DP:AF	0/0	21	39997	0.6167
OAS1	5188	12	.	G	.	24.88	RefCall	F	GT:GQ:DP:AF	0/0	23	39979	0.5709
OAS1	5295	12	.	G	.	21.74	RefCall	F	GT:GQ:DP:AF	0/0	20	39716	0.6306
OAS1	5303	12	.	G	.	23.76	RefCall	F	GT:GQ:DP:AF	0/0	24	39732	0.7674
OAS1	5331	12	.	T	.	20.6	RefCall	F	GT:GQ:DP:AF	0/0	25	39727	0.7325
OAS1	5381	12	.	T	.	24.35	RefCall	F	GT:GQ:DP:AF	0/0	22	39879	0.6201
OAS1	5415	12	.	A	.	25.13	RefCall	F	GT:GQ:DP:AF	0/0	7	39878	0.6779
OAS1	6220	12	.	T	.	22.34	RefCall	F	GT:GQ:DP:AF	0/0	21	39909	0.6466
OAS1	6221	12	.	G	.	7.54	RefCall	F	GT:GQ:DP:AF	0/0	27	40066	0.9081
OAS1	6305	12	.	G	.	21.58	RefCall	F	GT:GQ:DP:AF	0/0	23	40084	0.914
OAS1	6996	12	.	A	.	27.19	RefCall	F	GT:GQ:DP:AF	0/0	28	40100	0.9094
OAS1	7068	12	.	T	.	23.26	RefCall	F	GT:GQ:DP:AF	0/0	18	40104	0.3827
OAS1	7226	12	.	T	.	28.62	RefCall	F	GT:GQ:DP:AF	0/0	27	88190	0.6962
OAS1	7426	12	.	T	.	18.09	RefCall	F	GT:GQ:DP:AF	0/0	7	88187	0.5064
OAS1	8580	12	.	A	.	27.65	RefCall	F	GT:GQ:DP:AF	0/0	31	88188	0.8845
OAS1	8642	12	.	T	.	7.7	RefCall	F	GT:GQ:DP:AF	0/0	16	48091	0.5118
OAS1	8651	12	.	G	.	31.14	RefCall	F	GT:GQ:DP:AF	0/0	1	48011	0.4647
OAS1	8858	12	.	A	.	16.35	RefCall	F	GT:GQ:DP:AF	0/0	22	48000	0.8623
OAS1	9246	12	.	At	A	1.43	LowQual	F	GT:GQ:DP:AF	0/1	4	17	0.4118
OAS1	9285	12	.	C	.	22.31	RefCall	F	GT:GQ:DP:AF	0/0	0	47901	0.1216
OAS1	9657	12	.	Gtt	G	0	LowQual	F	GT:GQ:DP:AF	1/1	12	39783	0.4579
OAS1	9900	12	.	A	.	13.1	RefCall	F	GT:GQ:DP:AF	0/0	13	47829	0.3258
OAS1	10215	12	.	A	.	21.17	RefCall	F	GT:GQ:DP:AF	0/0	21	47736	0.7466
OAS1	10225	12	.	A	.	14.8	RefCall	F	GT:GQ:DP:AF	0/0	14	47735	0.71
OAS1	10504	12	.	A	.	26.37	RefCall	F	GT:GQ:DP:AF	0/0	26	47700	0.9
OAS1	11183	12	.	C	.	16.48	RefCall	F	GT:GQ:DP:AF	0/0	16	47516	0.6279
OAS1	11419	12	.	G	.	23.25	RefCall	F	GT:GQ:DP:AF	0/0	23	47492	0.7912
OAS1	11756	12	.	T	.	22.34	RefCall	F	GT:GQ:DP:AF	0/0	22	47424	0.6812
OAS1	12119	12	.	C	.	25.97	RefCall	F	GT:GQ:DP:AF	0/0	25	47319	0.6838
OAS1	13100	12	.	A	.	20.9	RefCall	F	GT:GQ:DP:AF	0/0	20	47301	0.5085
OAS1	13688	12	.	C	.	23.23	RefCall	F	GT:GQ:DP:AF	0/0	23	47287	0.7974
OAS1	13891	12	.	A	.	16.86	RefCall	F	GT:GQ:DP:AF	0/0	16	47292	0.4085
OAS1	13931	12	.	A	.	24.4	RefCall	F	GT:GQ:DP:AF	0/0	24	47304	0.7617
OAS1	13958	12	.	G	.	28.01	RefCall	F	GT:GQ:DP:AF	0/0	28	47300	0.8784
OAS1	14315	12	.	G	.	6.03	RefCall	F	GT:GQ:DP:AF	0/0	6	47275	0.5274
OAS1	15045	12	.	A	.	24.98	RefCall	F	GT:GQ:DP:AF	0/0	24	47019	0.6028
OAS1	15055	12	.	T	.	19.7	RefCall	F	GT:GQ:DP:AF	0/0	19	47016	0.4589
OAS1	15178	12	.	G	.	26.65	RefCall	F	GT:GQ:DP:AF	0/0	26	46915	0.7523
OAS1	15228	12	.	A	.	7.62	RefCall	F	GT:GQ:DP:AF	0/0	7	46681	0.5185

The quality of each SNP and the reference allele is shown. The information regarding whether each SNP is a heterozygote or a homozygote, is shown as 0/1 for heterozygote reference allele, 1/1 for homozygote for the alternate allele and 0/0 homozygote for the reference allele. If a particular SNP, has a “Pass” it most likely succeeded in the quality check, if it shows “Low Qual”, the SNP did not pass the quality check. The information in the last 3 columns is extra additional VCF information regarding each SNP.

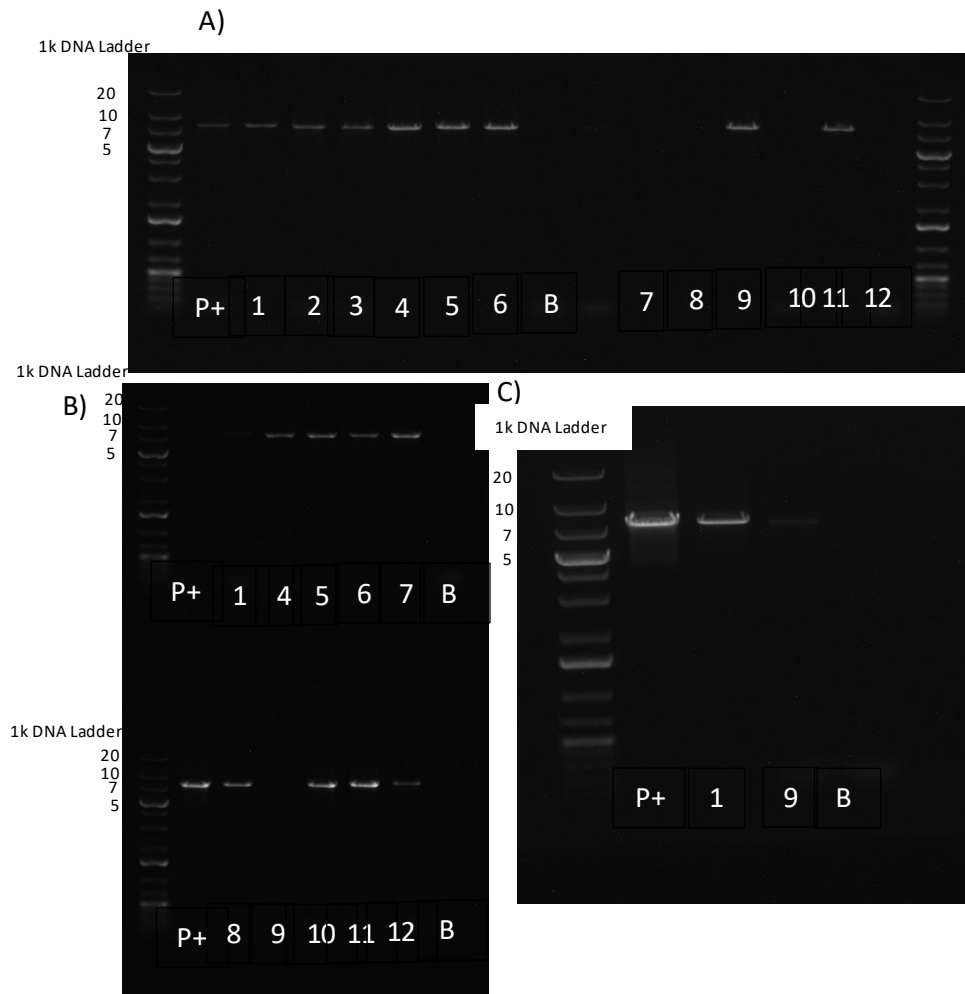


Figure 6 A visualisation of gel electrophoresis, amplicons produced via long-range PCR for all 12 samples from amplicon B. (A) shows a successful amplification of the desired fragments from individual samples 1 to 6. 7,8,10 and 12 were unsuccessful in the production of amplicons. (B) and (C) are gels from subsequent long-range PCRs performed to generate brighter amplicons, as well as produce amplicons for samples that did not work the first time. P+ is the positive control and B is the blank used. A 1k ladder was used, represented on the left of each gel.

Table 6- LD (r^2 value) between SNPs A and SNP B present in OAS1 with the RefLoc and rsID.

SNPA			SNPB			R2 value
RefLoc	Chromosome (Chr12: GRch38)	rsID	RefLoc	Chromosome (Chr12: GRch38)	rsID	
50	112905123	rs1371602093	73	112905146	-	0.333333
73	112905146	-	74	112905147	rs1316166758	1
74	112905147	rs1316166758	81	112905154	-	1
74	112905147	rs1316166758	82	112905155	-	1
81	112905154	-	88	112905161	rs565073789	0.641026
165	112905238	-	180	112905253	rs1301894944	0.25
179	112905252	rs2043282193	180	112905253	rs1301894944	1
180	112905253	rs1301894944	201	112905274	-	1
180	112905253	rs1301894944	214	112905287	rs1450155631	0.25
214	112905287	rs1450155631	241	112905314	-	0.75
214	112905287	rs1450155631	256	112905329	-	1
225	112905298	rs769428873	256	112905329	-	0.25
225	112905298	rs769428873	262	112905335	-	0.333333
241	112905314	-	275	112905348	rs984071445	1
7227	112912300	rs75344264	7426	112912499	-	1
8642	112913175	rs2285934	9246	112914319	-	1
14315	112919388	rs10774671	15228	112920301	rs7135577	1
14331	112919404	rs1131476	15228	112920301	rs7135577	1

LD data was generated using a combination of Plink and bcftools, to analyse a compiled vcf file taken from each of the samples. Blanks represent variants that are not present in dbSNP database.

Table 7- Number of samples with identified SNPs from the OAS1 locus observed in their respective groups, as homozygous or heterozygous variations represented by the genotype

Chr12:GRch38	rsID	Groups								
		1			2			3		
		0/0	0/1	1/1	0/0	0/1	1/1	0/0	0/1	1/1
112905118	rs898522467	0	1	0	0	0	0	0	1	0
112905119	rs995440229	0	0	0	0	2	0	0	1	0
112905123	rs1371602093	0	1	3	0	0	2	0	3	1
112905147	rs1316166758	0	2	0	0	0	0	2	0	0
112905152	rs2043281011	0	1	0	0	1	0	0	1	0
112905160	rs758866723	0	1	0	0	1	0	0	1	0
112905161	rs565073789	1	3	0	0	2	0	0	2	0
112905178	rs1468494281	0	3	0	0	0	0	0	1	0
112905179	rs2043281382	0	3	0	0	2	0	0	1	0
112905210	rs2043281756	3	0	0	1	1	0	1	0	0
112905242	rs925000289	0	2	0	0	1	0	1	0	0
112905251	rs532440053	1	0	0	0	1	0	0	0	0
112905252	rs2043282193	1	1	0	0	0	0	0	0	0
112905253	rs1301894944	0	1	2	0	0	1	1	0	2
112905287	rs1450155631	0	2	0	0	1	0	1	0	0
112905298	rs769428873	0	2	1	0	0	2	0	0	2
112905336	rs2043284068	0	0	0	0	0	0	0	1	0
112905338	rs2043284101	0	1	0	0	1	0	0	1	0
112905348	rs984071445	0	0	0	0	1	0	0	0	1
112905360	rs911137212	0	2	0	0	2	0	0	4	0
112906341	rs1015542	0	0	0	0	1	0	0	0	0
112907362	rs7956880	0	1	0	0	0	0	0	0	0
112907451	rs10744785	0	0	1	0	0	0	0	0	2
112907894	rs4766662	0	1	1	0	0	1	0	1	3
112910344	rs138617415	0	0	0	0	0	0	0	1	0
112910856	rs34137742	0	1	0	0	0	0	0	0	0
112911065	rs1131454	0	0	1	0	0	0	0	0	1
112912028	rs55676265	1	1	0	0	0	0	0	0	0
112912300	rs75344264	0	2	0	0	0	0	1	0	1
112913715	rs2285934	0	0	2	1	1	0	0	0	2
112914354	rs4767023	0	0	1	0	1	0	0	0	0
112914903	rs45495393	0	2	0	0	1	0	0	1	0
112917552	rs148741042	0	0	0	0	1	0	0	0	0
112918230	rs60623134	0	1	0	0	0	0	0	1	0
112919388	rs10774671	0	0	4	1	0	0	0	0	0
112919404	rs1131476	0	0	1	0	1	0	0	0	0
112919432	rs1051042	0	0	4	0	0	0	0	0	4
112920301	rs7135577	0	0	4	1	1	0	0	0	4

0/0 represents homozygous for the ancestral allele. 0/1 represents heterozygous for the alternate allele and 1/1 represents homozygous for the alternate allele. These are all the known SNPs seen in each group.